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NOTICES

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Assistant Editor: M. O'Connor, M.Sc.
Department of Scientific and Industrial Research, Wellington

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USE OF COPPER SULPHATE IN DIPPING SHEEP AND ITS EFFECT ON THE SUBSEQUENT PROCESSING OF THE WOOL

By L. F. STORY, New Zealand Woollen Mills' Research Association, Dunedin.

(*Received for publication, 17 June 1952*)

Summary

Copper sulphate is used by the sheepfarmer at three levels of concentration for treating sheep. Some of the copper is retained by the wool and may lead to faulty dyeing, as many chrome dyes are sensitive to the presence of copper, or to general discolouration due to the formation of copper sulphide.

There appears to be little objection to the use of copper sulphate at a very low concentration in gammexane dips, or at a high concentration for foot-rot baths, provided the fleece is not splashed. The use of an intermediate concentration for dipping against mycotic dermatitis is permissible to a limited extent, but any considerable use in this way is undesirable from the point of view of the wool user.

INTRODUCTION

Copper is well known as an unwelcome contaminant of wool during processing, as it causes stains, interferes in bleaching and affects the colours of many chrome dyes. Copper sulphate is also well known as a cheap and effective fungicide. It is used by the sheep farmer for three purposes:

(1) As a bacteriostat in gammexane dips.

(2) As a preventative of mycotic dermatitis, a skin disease similar to ringworm (Bull, 1929).

(3) As a "foot-rot" bath.

In view of the increasing use of "bluestone" by the farmer, it was of interest to find how much copper could be tolerated when it was actually present on the raw wool before processing commenced, and how much residual copper might be expected to be present on the wool when sheep have been treated with bluestone.

The Effects of Copper in Processing

(a) *Dyeing*: Copper interferes in the application of chrome dyes by forming a copper lake instead of the desired chrome lake. As the copper lake is frequently of a different colour the resulting dyeing is off-shade, the degree of off-shade depending on the relative amounts of copper and dye unless there is an excess of copper. Further, it has been shown by Millson (1949) that the copper lake is frequently less fast to washing and to light than the chrome lake.

Samples of wool containing known small amounts of copper were prepared by immersing scoured white fingering yarn in very dilute solutions of copper sulphate (5 g wool/50 ml), when nearly all the copper was rapidly taken up. The copper-containing yarns were dyed with a few selected chrome dyes and the resulting shades compared. Some typical results were as follows:

Solochrome Brown M, dyed metachrome:
0.5% markedly off-shade with 0.006% Cu;
completely off-shade with 0.01% Cu or with Cu metal.
1.5% just visibly off-shade with 0.006% Cu;
appreciably off-shade with 0.01% Cu or with Cu metal.
3.2% (standard depth) no effect with 0.006% Cu;
just visibly off-shade with 0.01% Cu and with Cu metal.
Solochrome Dark Blue B (C.I. 202), dyed afterchrome:
0.5% same as Solochrome Brown M.
2.0% just visibly off-shade with 0.006% or 0.01 Cu.

The results thus showed that even 0.006 per cent of copper was sufficient to have a marked effect on light dyeings of copper-sensitive dyes. Many other chrome dyes would be affected when used under similar conditions. For example, Millson (1949) lists 30 dyes which he found to be off-shade (using one per cent of each) when 1 p.p.m. of copper was present in the water used. As the liquor ratio was 60 : 1, this corresponds to 0.006 per cent copper on the weight of the wool.

In practice, the distribution of the copper must also be taken into consideration, as the copper on the laboratory-prepared samples was evenly distributed, whereas most of the copper on dipped wool is concentrated towards the tip end. Thus, although the average copper content may be only 0.005 per cent, the tip end may contain 0.02 per cent or more; this will cause marked "tippiness" in dyeing instead of a general off-shade effect.

(b) *Peroxide Bleaching*: Copper has a marked catalytic effect on peroxide bleaching. When samples of copper containing yarn were treated with solutions of hydrogen peroxide the loss of peroxide increased from 18 per cent with no added copper to 48 per cent with 0.07 per cent copper, and the wool was noticeably discoloured instead of being bleached.

(c) *Development of Stains*: Wool containing appreciable amounts of copper is noticeably stained green or has a greenish cast, but the amount of copper necessary for its presence to be detected in this way is several times greater than the quantity that will affect the shade of dyes. However, if the copper is converted into copper sulphide, a

smaller amount becomes evident owing to the development of a brown discolouration. Traces of hydrogen sulphide sufficient to do this are formed when wool is heated under damp conditions, e.g., in dyeing, tentering or blowing. Thus white wool containing small amounts of copper is liable to be discoloured during processing, while light dyeings are liable to be saddened even if the dyes are not affected directly by the copper. It was found that 0.01 per cent copper was sufficient to cause a perceptible darkening of shade of wool boiled for a short time, as compared with a control.

(d) *Effect of Metal-sequestering Agents*: Ethylene diamine tetra-acetic acid (E.D.T.A.) is effective in the dyebath in removing traces of undesirable metals derived from the machine, or in the water used, but appears to be relatively ineffective in sequestering copper which is already present on the wool, unless a large excess is used and only small amounts of copper are present. The position is aggravated in the case of copper-dipped wool by most of the copper being present towards the tip ends, as discussed earlier. Thus, while the E.D.T.A. would probably be effective against 0.005 per cent of evenly distributed copper, it is less effective against a local high concentration.

From these tests it appears that less than 0.006 per cent of copper can be tolerated if the wool is to be dyed in a light shade with a copper-sensitive dye (including the use of a small percentage of such a dye in a mixture). No actual maximum can be stated as the amount varies according to the nature and quantity of the dye used. In some cases no copper at all can be tolerated, and the presence of variable traces of copper derived from machinery may affect the shade.

QUANTITIES OF COPPER RETAINED BY WOOL AFTER DIPPING

Clean wool rapidly takes up copper from a dilute copper sulphate solution, presumably in electrovalent union with carboxyl groups. Greasy wool was found to take up considerably less copper in a laboratory experiment, since the grease forms a protective layer over the fibres and hinders access of the aqueous solution. In practice the copper taken up by the wool will probably be little more than that contained in the liquor retained by the fleece after draining, and which will be dried on to the wool. This is confirmed by the results of some analyses of gammexane-copper-sulphate (0.03 per cent) dipping baths in which the copper concentration was found to remain practically unchanged during dipping while the volume of liquid progressively decreased.

The suint of greasy wool contains acidic compounds (as potassium or sodium soaps) which react with copper sulphate to form insoluble copper compounds which are retained on the wool fibres. The copper on greasy wool is thus present in two forms, viz. part combined with the wool molecule and part combined with the acidic suint components. When the wool is scoured, most of the latter fraction is removed along with the grease while the former fraction would be expected to be unaffected. It was in fact found by experiment that a large proportion—roughly two-thirds—of the copper present in greasy wool was removed when

the wool was scoured or extracted with carbon tetrachloride, while the remainder resisted further scouring or extraction.

As it is only the copper remaining after scouring that is of significance in the mill, e.g., in dyeing, etc., samples were normally scoured before analysis, using 0.2 per cent Lissapol N (I.C.I. Ltd.) and 0.5 per cent salt at 50°c. The copper content was determined by digesting a sample of the scoured wool with a mixture of perchloric (60 per cent, 2 ml) and nitric (concentrated, 6 ml) acids. The digestion mixture was diluted and made alkaline, and the copper complex of dithio-diethylcarbamate formed in presence of citrate. The complex was extracted with carbon tetrachloride and the intensity of colour of the extract determined with a "Spekker" absorptiometer which had been calibrated by using known amounts of copper. The results of the analyses are given below as parts per million of scoured air-dry wool (approx. 13 per cent regain).

Practical Trials

Copper sulphate is used by the sheep farmer at three levels of concentration, viz.

10 per cent or more in foot-rot baths,
0.2 per cent (recommended) against mycotic dermatitis,
0.03 per cent in gammexane dips.

Foot-rot Baths and Troughs

The solution is usually put in a long, narrow, shallow trough through which the sheep have to walk single file. Contamination of the wool with copper may occur through splashing or through the sheep falling. Analysis of a sample of wool stained from a foot-rot bath showed the presence of 3 per cent of copper sulphate on the greasy wool, which is obviously far in excess of the maximum that can be tolerated under any circumstances.

Seven Corriedale sheep were dipped in copper sulphate solutions (0.05, 0.1 and 0.2 per cent) in May 1951 at Lincoln College. The sheep had also been dipped in gammexane and copper sulphate (0.03 per cent) in January. Samples of the wool were taken from hip and belly after approximately 1, 3 and 6 months. The results of the copper analyses on the 6 months samples (at shearing) are given in Table I. Most of these samples showed the presence of copper by having a greenish tinge.

TABLE I. COPPER CONTENTS OF SHORN SAMPLES FROM LINCOLN COLLEGE

Sheep No.	Conc. of $CuSO_4$ (%)	Amount of Cu (in p.p.m.) on Scoured Wool				
		Hip	Shoulder	Belly	Back	Rump
M 161	0.2	376 VG*	316 G*	376 G	311 g*	721 VG
M 312	0.2	253	278	84		
M 656	0.2	425 G	199 G	206	357 G	412 VG
M 544	0.1	204 G	208	89		
M 39	0.1	134	144	160 g	126	
M 40	0.05	175 g	154 g	41		
M 409	0.05	88	94	53	103	

* g = slightly green
G = green
VG = very green

The results were reasonably consistent as between the different sampling times but showed considerable variations from sheep to sheep, and between different parts of the same sheep, the belly samples usually containing much less copper than the hip or shoulder.

The means of the hip and belly samples over the two or three sheep in each group are given in Table II, together with the ratios to the 7 months' samples for each concentration. If no loss of copper had occurred through weathering, the ratios expected would be approximately 1.0 : 0.77 : 0.57, assuming uniform wool growth (seasonal differences would make the ratios rather higher than these). Thus, the indication is that losses due to weathering are not very large, so that most of the copper taken up by the wool is retained though the overall percentage decreases due to the inclusion of the later-grown wool.

TABLE II. MEAN COPPER CONTENTS OF HIP AND BELLY SAMPLES

Conc. of $CuSO_4$ (%)	Cu content (in p.p.m.) of samples at age indicated			Ratio of Cu content to that of 7-mth. sample		
	7-mth.	9-mth.	12-mth.	7-mth.	9-mth.	12-mth.
0.2	653	451	286	1.0	0.69	0.44
0.1	296	236	147	1.0	0.80	0.50
0.05	200	136	89	1.0	0.68	0.45
			Mean ratio	1.0	0.72	0.46

The copper contents of the 12 months' samples are still very high, and even the samples from sheep dipped at the lowest concentration (0.05 plus 0.03 per cent) contained a mean of 90 p.p.m. (0.009 per cent), which has been shown to be more than enough to affect many chrome dyes.

From the above results it would be expected that the amount of residual copper would be roughly proportional to the length of staple when dipping. If the sheep were dipped "off the shears," lower final copper contents would be expected.

The conclusion drawn from these results is that it is undesirable from the point of view of the wool user to dip sheep in copper sulphate solutions, except possibly when dipping is done soon after shearing and with a maximum concentration of 0.2 per cent. Dipping at this time would be of value in clearing up an existing infection but would probably be of little use in inhibiting the development of a new infection in the following autumn. In any case experience has been that shorn sheep are little affected, and that it is usually the hoggets which contract the disease; it has been suggested by Bull (1929) that the latter should be dipped in March as a precautionary measure.

Gammexane Dip containing Copper Sulphate

In this dip which is now widely used in New Zealand as a standard dip within the meaning of the Stock Act 1908, the copper sulphate is present as a bacteriostat so that a standing bath can be maintained and topped up as required. In view of the appreciable

amounts of copper found in samples from Corriedale sheep at Lincoln College that had been dipped in May with 0.05 per cent copper sulphate and in January with 0.03 per cent copper sulphate plus gammexane, tests have been made on a variety of samples to find how much was present after dipping with gammexane plus copper sulphate only, or a non-copper-containing dip.

Twelve samples from the Lincoln College Corriedale sheep contained appreciable amounts of copper, ranging from 24 to 75 (mean 45) p.p.m. Samples from an early-shorn Corriedale clip also contained from 38 to 78 p.p.m. A considerable number of other samples contained up to 18 p.p.m. with the exception of one hogget wool (28 p.p.m.).

As small quantities of copper of the order of 10 to 20 p.p.m. are likely to be picked up during normal processing in the mill, the small quantities found to be derived from gammexane dips are unlikely to cause any additional trouble except in a few cases. The largest amounts are to be expected on early shorn wools dipped in April when the sheep are carrying about 8 months' wool.

THE ECONOMIC SIGNIFICANCE OF THE RESULTS

Gammexane Dips

The results of the tests indicate that in general the amount of copper present on the wool after shearing and scouring is small enough to be neglected. Thus, although a large proportion of the clip is affected, it is unlikely that any trouble would be experienced, and there is no justification for stopping the use of copper sulphate in gammexane dips provided the present concentration of 0.03 per cent is not exceeded.

Foot-rot Baths

"Bluestone" is used universally for this purpose at a high concentration. If the fleece is splashed an intense but local stain is produced which will probably cause the lot to be penalised. However, this stain is actually less of a liability in the mill than a small amount of copper evenly distributed and invisible, since the stain stands out and the affected wool can be sorted out and put with other stained wool for dyeing black. The avoidance of staining can be achieved by careful handling of the sheep when treating them.

Dipping Against Mycotic Dermatitis

The use of copper sulphate at a relatively low concentration (0.2 per cent) has a more insidious result. The greasy wool may not be stained so that the wool passes through the sale and the mill sorting-room unsuspected, and the presence of the copper is not detected until the unfortunate dyer tries to dye it with a light shade of a sensitive chrome colour.

The economic seriousness of the fault depends on the quantity of wool likely to be affected and on how the wool happens to be handled at the mill. Thus, if only the hoggets in a few clips have been dipped, the copper content resulting will probably be of little

consequence since the wool is likely to be mixed with much other wool low in copper. The average copper content will thus be reduced enough for it to be harmless. However, if on account of a bad season or for other reasons an appreciable proportion of the total clip were to be dipped, then the copper content might not be reduced sufficiently by dilution with other wool, and off-shade and tippy dyeing would result. Two other points may make the copper content significant: first, the copper-affected wool from a large clip might go through the mill as a single batch (or with not much dilution) so that the effect of the copper would be observed; secondly, as it is hogget wool that would most likely be affected, it is quite conceivable that even though hogget wools from a number of clips were used in a batch, all or most of the clips might be affected and the resulting blend would still have a considerable copper content.

CONCLUSIONS

(1) Many chrome dyes are affected (in light shades) by very small quantities of copper on the wool.

(2) When 0.03 per cent copper sulphate is used in a gammexane dip the final copper content of the wool after scouring is usually small enough to be neglected. This practice is therefore not contra-indicated.

(3) 0.2 per cent copper sulphate has been recommended as a dip against mycotic dermatitis and appears to be beneficial. However, the resultant copper content of the wool is likely to be well above the maximum that can be tolerated in case the wool is dyed with a copper-sensitive dye. While there would be less objection to this dipping on a limited scale, since the affected wool would probably be mixed with other copper-free wool which would reduce the average copper content, any considerable use of copper sulphate at this concentration would be undesirable from the point of view of the wool user, as it would lead to obscure and annoying dyeing faults and dingy whites.

(4) There is no direct objection to the use of concentrated solutions of copper sulphate as foot-rot baths since the solution should only be applied to the sheep's feet. If the solution also reaches the fleece an intense stain is produced and the wool is likely to be penalised by the buyers.

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SURVEY OF LOSSES IN PIGS ON NEW ZEALAND FARMS, 1949/51.

By A. LONGWILL, Animal Industry Division, Department of Agriculture, Wellington

(*Received for publication, 23 January 1952*)

Summary

(1) A report is given of the results of a survey of losses in pigs in 4169 litters from a sample of New Zealand dairy farms reasonably representative of those on which the bulk of New Zealand's pigs are produced, during 1949/50 and 1950/51.

(2) The number of pigs born per litter (9.0) appears to be much less variable than the number born per sow per year (14.7 in 1950/51 as against 16.3 the previous year). The influence of sow nutrition on litter frequency, coupled with the use of inherently prolific sows is pointed to as fundamental to the achievement of a high figure for output of pigs per sow.

(3) Losses to weaning averaged 24.01 per cent of total pigs born. The necessity for investigation of possible disease aspects and the desirability of providing a brooder in farrowing houses is indicated.

(4) There is a nett increase in the number of pigs weaned per litter up to 13 born per litter. Of the breeds, the Large Whites is outstanding in prolificacy, but losses are also higher in this breed and tend to negative the initial advantage.

(5) As compared with overseas figures, New Zealand sows on the whole are less prolific, losses through stillbirths are apparently higher and, taking into account the greater risks associated with higher prolificacy, the overall percentage loss to weaning, although comparable with those of overseas countries, is not satisfactory.

(6) Post-weaning losses, although only 3.6 per cent of the total born, are nevertheless a serious economic loss. An evaluation is made of the financial losses involved to the industry generally,

INTRODUCTION

The survey described in this paper was undertaken on the recommendation of a Technical Advisory Committee set up by the National Pig Council Executive in 1948. The Committee, after a careful examination of the position came to the conclusion that the most fundamental problem facing the pig industry was the small number of pigs slaughtered each year per sow carried. It considered that possible contributing factors were:

(1) Low fertility of breeding stock;

(2) Small number of litters per sow in a season;

(3) Heavy losses among pigs on the farm.

It was suggested that these factors should be investigated concurrently and that a survey on a Dominion basis would be required to obtain an accurate picture of the incidence of losses.

A survey was carried out on a sample of New Zealand dairy farms reasonably representative of those on which the bulk of pigs are produced.

SUMMARY OF PREVIOUS LITTER RECORDING RESULTS

Information already available from the records of the pig recording clubs which operated in New Zealand in the early thirties and from Department of Agriculture official sow recording indicated that sows were generally not very prolific as compared with those in Denmark, Canada or the United Kingdom, and that there was substantial mortality in litters, particularly in the day or two immediately after farrowing.

The first pig recording work in New Zealand in 1928/29 revealed a 20 per cent mortality from birth to weaning[1]. Analysis of the records of 467 litters recorded by the Waikato Pig Recording Club in the years 1932/33 and 1935/36 showed that the average number of pigs born per litter was 9.3, and that 7.3 were weaned, showing a loss of 22.7 per cent up to weaning stage. Of the 4,414 pigs born, 974 perished before weaning stage. Records showed that of the pigs lost, 43.3 per cent were overlain, 32 per cent were born dead, 12.3 per cent died at birth, while 8.0 per cent were either runts or "bad doers" which subsequently died or were destroyed. Other causes accounted for the remaining 4.4 per cent.

Similar figures for the Manawatu Recording Club for the years 1933 to 1935 and covering 4,190 piglets born, of which 835, or 20 per cent, were lost before weaning, showed 48 per cent of the total was due to pigs having been born dead or having died at birth, 42 per cent to pigs being overlain, while 10 per cent loss was due to runts and other causes. The loss, based on pigs born alive, was 11 per cent[2]. Other figures for break-up of losses to weaning according to causes present a similar picture[3].

Both these sets of figures indicate that approximately 90 per cent of the losses to weaning occur at, or within a short time after, farrowing, and point to the importance of personal attention at farrowing time if these losses are to be avoided.

Department of Agriculture official litter records covering 1,325 litters and 12,411 pigs born, indicate a loss between birth and weaning of 20.6 per cent of total births. The percentage loss is not reduced to the extent which might have been expected in view of the fact that sows nominated for official litter recording were probably given special management.

Data from the pig census collected by the Department between 1938 and 1943 and involving 49 farms and 230 breeding sows, showed a rising tendency in percentage losses. In 1943 returns, there was an average of 1.5 litters per sow in the 12 months' period.

SURVEY TECHNIQUES USED

The survey was commenced in 1949, and an interim report covering the 1949/50 season was presented to the National Pig Industry Council in December, 1950.

For reasons of organization and economy every county was not included in the sample, but care was taken to ensure a reasonable geographic distribution, and an equitable distribution according to the number of sows and the intensity of pig production in order to give proper representation of the various feeding practices throughout the country. The counties were first arranged in groups according to the number of breeding sows in 1947. These groups comprised counties with 0-500, 500-1,000, 1,000-1,500, and more than 1,500 sows. These groups were further subdivided in accordance with the number of pigs per 100 dairy cows in milk, and also in North-South geographical order. The counties were then drawn so as to be representative without loss in statistical accuracy. Farms were grouped according to the

number of sows, e.g. 1-2, 3-5, 6-9, 10-15 and over 15. The use again of the variable sampling fraction provided the farms which were to be the basis of the survey.

The survey was repeated in the 1950-51 season over a different sample of farms. In the first season's work, records from 277 farms out of a total of 688 which had commenced keeping records were all that were available for analysis. Since it was felt that this meant that an automatic selection of the better farms had taken place, it was decided to base the new sample on a compact group, such as a dairy factory supply cream run or such other convenient area, and to collect records from all pig-producing farms in that area. Unfortunately, there was just as great a proportionate loss in the returns on the second occasion as in the earlier survey, records from only 155 farms being available for analysis. The final number of farms used, representing overall only 29 per cent of the original sample and 41 per cent of the second sample was disappointing. It can only mean that an automatic sorting of the material has taken place, the less efficient 66 per cent of the farms having fallen out. Any statistical analysis of the results is, therefore, out of the question. All that can be done is to analyse the data and report on this as being from a better-than-average sample of farms.

Two summaries were prepared from the shed sheets, one on a sow basis, and one on a farm basis. From these summaries the data was carded and totalled on Powers Samas calculating machines.

In the following tables, the data worked on a farm basis refers to complete returns from the 277 farms in 1949-50, and 155 farms in 1950-51.

Number of Sows and Cow/Sow Ratio

The average number of sows, calculated from the number on hand at the beginning and end of the recording period respectively, with number of cows milked, was used to calculate the ratio of cows to sows as shown in Table IV.

Table I. District Totals of Cows and Sows on Survey Farms and Ratio of Cows to Sows (expressed as Cows per Sow)

District	No. of Farms	No. of Cows	Number of Sows			Ratio Cows/ Sows
			At Beginning	At End	Average	
Northland	17	917	56	73	64.5	14.2
Auckland	13	647	64	57	60.5	10.7
Waikato	22	1,467	104	109	106.5	13.8
Bay of Plenty, including Tairawhiti (1)	25	2,294	180	181	180.5	12.7
Taranaki	23	1,412	99	105	102.0	13.8
Wellington	17	928	93	102	97.5	9.5
Canterbury	18	630	99	99	99.0	6.4
Otago/Southland	20	1,467	156	153	154.5	9.5
1951	155	9,762	851	879	865	11.3
1950	277	14,481	1,475	1,467	1,471	9.8
Grand Total	432	24,243	2,326	2,346	2,336	10.4

The farms covered were typical of those in which the dairy by-products are fully utilised by pigs.

In all districts the ratio of cows to sows is much lower than the Dominion average which, for some years, has been in the region of 25 cows to one sow. This contrast supports the conclusion that the farms in this sample are considerably above average in the use of their dairy by-products through pigs. On individual farms, however, there is an extremely wide range of variation in the ratio of cows to sows, e.g., from 4 : 1 to 70 : 1.

RESULTS

Table II shows the total, by districts, of litters for which the records of losses up to weaning were recorded satisfactorily for use in this analysis, together with the total number of pigs recorded born, the average number born per litter and the average number born per sow per year.

TABLE II. TOTAL PIGS BORN AND AVERAGE SIZE OF LITTER

District	No. of Litters	No. of Pigs Born	Average per Litter	Average per Sow per Year
Northland	110	951	8.6	14.7
Auckland	97	960	9.9	16.0
Waikato	187	1,546	8.3	14.6
Bay of Plenty	267	2,331	8.7	12.9
Taranaki	202	1,963	9.7	19.2
Wellington	157	1,379	8.8	14.2
Canterbury	156	1,420	9.1	14.4
Otago and Southland	239	2,259	9.5	14.7
1951	1,415	12,809	9.1	14.7
1950	2,754	24,834	9.0	16.3
Grand Total	4,169	37,643	9.03	15.8

Number of Pigs Weaned

Table III shows the Dominion Figures for the number of pigs weaned per litter and per sow for the years 1950-51.

TABLE III. PIGS WEANED PER LITTER AND PER SOW PER YEAR

District	No. of Litters	Total Pigs Weaned	Av. No. Weaned per Litter	Av. No. of Litters per Sow per Year	Av. No. Pigs Weaned per Sow per Year
1951	1,415	9,585	6.8	1.64	11.1
1950	2,754	19,019	6.9	1.80	12.5
Grand Total	4,169	28,604	6.9	1.77	12.2

It is apparent from Tables II and III that the number of pigs born and weaned per litter varies less than the regularity of breeding as represented by the average number of litters per sow per year. This is in line with the findings of Ballinger at Ruakura[4], who indicated that, while severe differences in nutritional level of breeding sows had little effect on numbers born per litter, poor feeding, as represented in New Zealand by grazing on burnt-up

pastures, with little or no supplementary feeding, resulted in lowering of the litter frequency. This points to a weakness in the typical method of running dry sows in New Zealand—grazing on general purpose mixed grass and clover pastures or, worse still, in pens where perennial ryegrass predominates.

Losses to Weaning

Over the whole survey, losses to weaning averaged 24.01 per cent of the total pigs born. Table IV give the analysis for 1950-51 season. A detailed analysis was not obtained in the previous year.

Table IV. Causes of Losses to Weaning in 12,809 Pigs Born

Cause of Loss	Number	Per cent of Total Born
Born dead	1,273	9.9
Died at birth	431	3.4
Overlain	897	7.0
Runts (died or destroyed)	163	1.3
Disease	142	1.1
Accidents or other causes	318	2.5
Total losses to weaning	3,224	25.2

It is not felt that the subdivision as between "Born dead" and "Died at birth" is of a high degree of accuracy. In a survey such as this, covering farrowings which in only few cases are observed, it will be more satisfactory to group the three major causes which account for the bulk of losses at, or immediately following, farrowing. It is interesting to note, therefore, that for the two years of the survey the total losses from these three causes represent a considerably higher percentage of the total pigs born than those recorded at Ruakura Research Station over a period of 5 years. In all litters covered by the survey 4,169 litters totalling 37,643 piglets, this percentage is 19.9 of piglets born; the corresponding figure at Ruakura where farrowings were observed and full details recorded of 1918 piglets born, was 15.9 per cent; this figure included 7.7 per cent born dead, 1.5 per cent died at birth and 6.7 per cent overlain; other causes brought the total loss in these litters to 21.5 per cent. This, however, is considerably lower than the total losses of 29.6 per cent recorded for all litters at the Station over 12 years.

Since losses in litters in other countries are frequently quoted on the basis of pigs born alive, it is of interest to note that from Table IV the loss to weaning is 1,951 of 11,536 piglets born alive, i.e. 16.9 per cent. If piglets which "died at birth" are included with those "born dead," it is seen that 1,520 piglets were lost out of 11,105 born with a chance of survival, i.e. 13.7 per cent.

Causes of Losses to Weaning

Details of stage or cause of losses are available for 2,117 litters comprising 19,725 piglets born; an average of 9.3 per litter. These litters are larger than the average for this survey (9 piglets). Losses are also considerably above average.

TABLE V. CAUSES OF LOSSES OF PIGLETS TO WEANING
(Total for 2 years)

Cause	No of Piglets Lost	Losses Expressed as % of Pigs Born			Losses Expressed as % of Losses to Weaning		
		Total	Excl. "Born Dead"	Excl. "Born Dead" and "Died at Birth"	Total	Excl. "Born Dead"	Excl. "Born Dead" and "Died at Birth"
Died at birth	1,982	10.0	—	—	36.7	—	—
Born dead	1,007	5.1	5.7	—	18.7	29.5	—
Overlain	1,645	8.4	9.3	9.8	30.5	48.2	68
Runts (died or destroyed)	251	1.3	1.4	1.5	4.7	7.4	10
Disease	191	1.0	1.1	1.1	3.5	5.6	9
Accident or some other cause	318	1.6	1.8	1.9	5.9	9.3	13
Totals	5,394	27.4	19.2	14.3	100.0	100.0	100

The figure of 10 per cent for still births (or, if "died at birth" are included, 15.7 per cent) is higher than those reported from overseas sources and closer investigation of causes would be useful. Overlying is by far the largest cause of loss of piglets born with a chance of survival, and this problem is one which should be made the subject of further research. Brooders, providing artificial heat, appear to be worthy of further trials.

Effect of Litter Size

Table VII sets out the pre-weaning losses in litters ranging from 6 to 17 piglets born taken out over 1,000 litters.

TABLE VI. LOSSES TO WEANING ACCORDING TO SIZE OF LITTER
(Based on random sample of 1,000 litters)

No. of Litters	No. Born in Litter	Total No. Weaned	Av. No. Weaned per Litter	No. of Losses to Weaning	% Loss to Weaning (Based on Total Born)
137	6 or less	566	4.13	115	16.8
119	7	686	5.76	147	17.6
148	8	983	6.64	201	16.9
155	9	1,128	7.26	267	19.1
144	10	1,134	7.87	306	21.3
113	11	967	8.55	275	22.1
92	12	815	8.86	285	25.9
38	13	355	9.34	142	28.5
21	14	179	8.53	115	39.1
20	15	164	8.20	138	45.7
7	16	75	10.71	37	33.0
6	17 or more	55	9.16	51	48.1
Total 1,000	Total born 9,186	7,107	7.11	2,079	22.6

Although the percentage loss increases with increase in fecundity of the sow, there is a nett increase in the number of pigs weaned per litter up to the level of 13 pigs born. Under present standards of weaning and management it would be worthwhile to

select for prolificacy up to this level but, until ways and means of preventing more of these losses are established, not to a higher level.

Effect of Breed of Sow and Boar

In the second year's survey, the breeding of sows and boars was recorded in a large proportion of cases; the results have been analysed on a breed basis covering 1,178 litters and 10,470 piglets born.

The average number born in this group was 8.9. Of purebred sows, the Large White, averaging 9.6, was the only breed better than average. Sows of the other pure breeds averaged approximately one pig less than the Large White in all cases. In the case of pure Large White sows, however, losses were almost 33 per cent and the number weaned per litter, viz. 6.5, was less than that for pure Berkshire sows, viz. 6.6, although it was better than for other purebred sows.

Crossbred sows including Large White as one parent in the cross were outstanding in prolificacy. Large White × Berkshire sows averaged 9.7 born and 7.9 reared, "Large White cross," 11.5 born and 7.5 reared. The only other group with prolificacy significantly above average was "Tamworth cross" with 9.6 born and 7.1 weaned.

Litters by different breeds of boar showed no significant differences in numbers born or percentage loss to weaning.

Effect of Age of Sow

The data from the 1950-51 survey was analysed to examine the trends in losses with increasing age of the sow. Table VII sets out the percentage loss for litters in rising sequence number. Lowest losses are recorded at 2nd and 3rd litters with a trend to increased losses thereafter. Average number born increases up to the 5th litter, but performance does not appear to improve after the third which shows the highest average number weaned.

Table VII. Effect of Age of Sow on Number of Pigs Born and Weaned

Sequence No. of Litter	No. of Litters	Pigs Born		Pigs Weaned		Loss to Weaning (%)*
		Total	Litter Average	Total	Litter Average	
1	197	1623	8.2	1200	6.1	26.1
2	189	1715	9.1	1339	7.1	21.9
3	169	1586	9.4	1245	7.4	21.5
4	154	1481	9.6	1082	7.0	26.9
5	133	1298	9.75	966	7.3	25.6
6 or more	258	2218	7.9	1677	6.5	24.4

* Based on total number of pigs born.

Effect of Type of Farrowing Accommodation

Losses to weaning have not been appreciably affected by the type of farrowing accommodation. There were no significant differences between results where farrowing took place in the best type of house and those where it took place out of doors. The use of artificial heat appeared to reduce losses but the number of litters in the category was too small to admit of definite conclusions being drawn.

TABLE VIII. RECORDS OF LOSSES OF PIGS TO WEANING RECORDED IN VARIOUS COUNTRIES
(Extracted from U.K. Bacon Development Board Bull. No. 4)

Source of Survey	No. of Litters	Pigs Born per Litter	Pigs Weaned per Litter	Per Cent Loss Birth to Weaning	Litters per Sow p.a.	Pigs Born per Sow p.a.	Pigs Weaned per Sow p.a.
GREAT BRITAIN:							
Kent, 1933-37	2,731	10.0	8.2*	18	1.6	16.0	13.1*
Hertfordshire, 1933-37	1,565	10.5	8.0	24	1.9	20.0	15.4
Staffordshire, 1936-37	290	10.1	7.0	31	—	—	—
N.P.B.A. Ped. Herds, 1930-36	61,118	10.5	8.0	24	—	—	—
GERMANY:							
Pomerania: Ordinary Herds, 1936	1,660	9.1	7.4	19	1.8	16.2	13.2
Bavaria, 1937	4,694	10.5	8.2	22	1.8	18.9	14.8
All Germany, 1934**	30,000	10.2	8.1	21	—	—	—
SWEDEN:							
English Ped. L.W.	18,955	10.8	8.6†	20†	1.7	18.4	14.6†
DENMARK:							
Breeding Centres, 1928-37	31,870	11.4	8.8	23	1.9	21.7	16.7
CANADA:							
Dom. Stats.	825,600	9.6	7.7	20	—	—	—
U.S.A.	—	—	6.2‡	29			Not over 10
NEW ZEALAND:							
Manawatu Pig Rec. Club	435	8.8	7.9	11	—	—	—

* Number per litter at 6 weeks.
** Approx. 1.5 per cent of total breeding sows.
† Number per litter at 3 weeks old.
‡ Pig Crop Report 1951 shows Av. of 6.65 for fall litters 1950 and 6.46 for spring litters 1951.

Comparison with Position in Other Countries

The percentage loss to weaning shown in the survey is comparable with that in other countries (see Table VIII)[6], as is also the number of litters per sow per year. However, prolificacy of New Zealand sows does not compare favourably with that of sows in overseas countries and points to the need for more prolific breeds and strains. As a progressively higher percentage loss to weaning may be expected with higher prolificacy, the position regarding losses in New Zealand also compares unfavourably with that in countries with more prolific breeding stock. When New Zealand's natural advantages as regards grazing range for breeding stock is taken into consideration, no room is left for complacency.

Post Weaning Losses

An analysis was made of post-weaning losses on the basis of stage of growth and season of the year. The months September-November inclusive were classed as "spring," December to February as "summer," and so on.

Thirty per cent of the post-weaning losses occurred during the spring months when the largest numbers of pigs were on hand, 29 per cent occurred during the winter quarter, 25 per cent in summer, and only 16 per cent in autumn.

Weaners and slips accounted for 79 per cent of the losses in spring, 77 per cent in autumn, 67 per cent in winter, but only 48 per cent in summer when porker-weight pigs accounted for 45 per cent of the loss. The heavy losses soon after weaning points to the need for more careful weaning management, better use of the creep in getting pigs well started on their own food before weaning, and more attention to the quality of the feed: overfeeding of bulky dairy by-products to newly-weaned pigs should be avoided. The heavy losses of porker pigs in the summer months is probably due to the higher incidence during the warm, humid weather, of Salmonellosis amongst pigs at this stage of growth.

Total Losses

Table IX summarises the whole losses data for the two seasons. Losses-to-weaning based on all piglets born (including stillborn) are 23.5 per cent, and total losses on the survey farms in these years, 27 per cent of pigs born. Post-weaning losses represent 4.7 per cent of pigs weaned.

TABLE IX. TOTAL LOSSES OF PIGLETS IN VARIOUS DISTRICTS

District	Piglets Born	Losses			Expressed as % of Piglets Born
		To Weaning	After Weaning	Total	
Northland	951	253	15	268	27.1
Auckland	960	257	27	284	29.6
Waikato	1,546	296	56	352	22.8
Bay of Plenty	2,331	675	199	874	37.5
Taranaki	1,963	573	54	627	32.0
Wellington	1,379	278	30	308	21.8
Canterbury	1,420	310	28	338	23.8
Otago and Southland	2,259	582	22	604	26.8
TOTALS: 1951	12,809	3,224	431	3,655	28.5
1950	23,936	5,408	885	6,293	26.3
GRAND TOTAL	36,745	8,632	1,316	9,948	27.0
Losses as % of Total Pigs Born		2.35	3.6		
Post-weaning Losses as % of Pigs Weaned			4.7		

EVALUATION OF ECONOMIC LOSS INVOLVED

Based on a sow population of 74,000 (1950 census) and on the data obtained from this survey, the total loss to weaning (3.6 pigs per sow per year) is of the order of 266,000 piglets. Similarly, the post-weaning losses, on the assumption that these farms are representative, would be of the order of 42,500 pigs.

This assumption would lead to the expectation of 860,000 pigs marketed, i.e. 11.6 pigs per sow. The actual marketing figures however are known to be slightly less than 10 per sow.

Therefore, the number of pigs born, weaned and marketed per sow is lower in the industry generally than revealed by this survey. This is almost centainly due to the fact that on most farms the reproductive rate is lower than on the farms covered in the survey.

At a very conservative estimate, the above mentioned losses represent over half a million pounds annually, as is seen from the following calculations:

266,000 piglets lost to weaning	@	£1 10 0	£400,000
42,500 weaners, etc.	@	£3 10 0	£150,000
			£550,000*

It could be argued that all these pigs could have been fattened on the food supply available if they had been saved; if valued half as porkers and half as baconers they would represent a monetary loss of over £2,000,000. The true loss probably lies somewhere between these two extremes. Whatever the figure, it is very substantial.

The loss or "leakage" at present represented by the inefficient use of sows can be estimated in the following way:—

Difference between number of pigs marketed on survey farms per sow and on Dominon average
= 1.9 pigs per sow.

On 74,000 sows, this represents 140,000 pigs

Value ½ pork : ½ bacon (Av. £6 10 0) = £910,000*

As ample supplies of food are available for fattening this extra number of pigs, it is apparent that better feeding and management of sows immediately after weaning their litters should give very valuable results. This probably applies with greatest force to sows weaning their litters between January and July and may account, in part, for the relatively small percentage of sows which are actually farrowed in the ideal months to suit the New Zealand food supply, viz. May-June and November-December.

Discussion

While it must be emphasised that, in both years, returns were received from farms where the management of and interest in pigs generally was somewhat above average, it is probable that the losses revealed are a true reflection of those throughout the industry. The fact that the average number of pigs sold per sow in New Zealand is lower than on the survey farms is more likely to be a result of the less efficient use of sows on below-average farms[4].

This survey confirms previous observations regarding losses to weaning and also the more restricted data from Pig Census returns of post-weaning losses[5]. In the second season, bought-in pigs were separately dealt with and a loss of 7.5 per cent of purchased pigs as against 4.7 per cent overall post-weaning loss emphasises the soundness of a home-breeding policy as a general rule.

Conclusions

The main conclusions to be drawn from this survey are:

(1) Of the total number of piglets born, some 23 per cent are lost at birth or within a few hours. These losses appear to be relatively little affected by any known factors in management. Good sow nutrition, insofar as it is responsible for increasing the weight of piglets at birth, can assist in reducing losses, but the method of ensuring this under New Zealand conditions has not been established.

(2) The low output of pigs per sow is a function of the low litter frequency and low fertility of New Zealand sows. Regular breeding can be assisted by feeding sows well after weaning each litter so as to ensure rapid regain of lost weight. To raise the general level of prolificacy, advantage should be taken of superior breed followed by progeny testing to maintain desirable characters.

(3) The provision of artificial heat in the farrowing house appears to offer hope of reducing pre-natal mortality.

(4) The relatively large numbers of pigs born dead or dying at birth, and the frequent failure of sows to breed are problems requiring the attention of veterinary research workers.

(5) In view of the high degree of uniformity for different districts of the number of pigs born and weaned per litter, future investigations on these points may be confined to a sample of farms in selected districts.

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* The cash values quoted on pages 303 and 304 applied at the time the paper was compiled—late 1951. Since then there has been a marked upward trend in values and as at time of publication prices for weaners are some 25 per cent higher, for porkers 35 per cent and baconers 20 per cent higher than the figures quoted.

CONTROL OF PRE-HARVEST DROP OF WASHINGTON NAVEL ORANGES

By R. M. Davison, Fruit Research Station, Department Scientific and Industrial Research, Auckland

(Received for publication, 1 May 1952)

Summary

Dilute aqueous sprays of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) were tested for control of pre-harvest drop of Washington Navel oranges at Kerikeri. A reduction in drop of 60-80 per cent was achieved with 2,4-D and 2,4,5-T at 20 p.p.m. but MCPA was less effective and gave only a 48 per cent saving.

A single application of 2,4-D in late autumn, either before or after fruit drop had commenced, reduced loss for periods up to fifteen weeks until harvesting in August or September.

The duration of effectiveness of 2,4-D was markedly reduced when mixed with Bordeaux or Cuprox sprays.

Applications were made in late autumn after shoot growth had ceased and no leaf curling occurred.

Introduction

Certain sweet orange varieties grown in New Zealand lose a proportion of their crop each year through pre-harvest or winter-drop of sound fruit. Drop commences in late autumn and continues throughout the winter until the crop is harvested in August or September. This annual loss may be considerable, especially with susceptible varieties in unfavourable seasons.

Stewart and others (1947) first reported in a series of papers the use of dilute 2,4-dichlorophenoxyacetic acid (2,4-D) sprays to reduce pre-harvest drop of grapefruit and oranges, and subsequently their effectiveness on citrus varieties in other countries has been demonstrated. Strengths recommended for commercial usage have varied from 8 to 20 parts per million (p.p.m.) 2,4-D. Foliage distortion in the form of curling of young leaves has been reported when actively growing trees were sprayed (Stewart and Klotz, 1947). These same authors found that higher concentrations of 2,4-D resulted in the formation of abnormal fruits.

The effectiveness of 2,4-D and similar chemicals in preventing fruit drop of Washington Navel oranges was tested in Kerikeri during 1949-51. Preliminary reports on these trials have already been published (Davison and Cederman, 1950, 1951) and detailed results are given below.

Experimental Procedure and Results

Mature Washington Navel orange trees on private orchards were used. In each experiment selected trees were grouped into randomised blocks giving five to eight single tree replications. All fallen fruits were removed from beneath trees immediately after spraying and fruits dropped thereafter were recorded at approximately weekly intervals. Except for one early trial, the total

fruit on each tree at the time of spraying was calculated by adding the number of fruits harvested to the number of fallen fruits. Figures for drop given below are for sound fruit only expressed as a percentage of fruit on the tree at the beginning of each experiment. Dropped fruits were classed as unsound if damaged by pest or disease attack or mechanical injury.

Close observation for possible foliage injury was made at intervals following application in all instances.

1949 Trials

Two separate experiments were conducted during the winter of 1949. In Experiment I sprays of 5 and 10 p.p.m. 2,4-D were applied to a block of trees on 2 June. These trees had received a copper spray two days previously and as the effect of copper residues on the hormone was not known, a further experiment was arranged on a block which had not received any recent fungicidal sprays. Treatments applied to this block on 3 June (Exp. II) were:

(1) Control—water spray

(2) 10 p.p.m. 2,4-D

(3) 10 p.p.m. 2,4-D + Bordeaux 3.4.50.

In both experiments 2,4-D powder was first dissolved in alcohol before mixing to facilitate solution. In preparation of the Bordeaux-hormone mixture 2,4-D was added to freshly mixed fungicide. Sprays on both orchards were applied by bucket pump in fine sunny weather. Some drop had occurred on both blocks prior to spraying.

Results are given in Table I.

TABLE I. EFFECT OF WATER SOLUTION OF 2,4-D ON PRE-HARVEST DROP OF WASHINGTON NAVEL ORANGES: 1949 TRIALS
(6 trees used for each treatment)

Treatment*	Average drop %
EXPERIMENT I	
Control: Water spray	10.6
2,4-D, 5 p.p.m.	10.0
2,4-D, 10 p.p.m	5.9
Difference for significance at 5% level	6.9
EXPERIMENT I	
Control: Water spray	5.2
2,4-D, 10 p.p.m.	3.8
2,4-D, 10 p.p.m. + Bordeaux 3-4-50	5.6
Difference for significance at 5% level	2.7

*Agral L (3 oz/100 gal) used with all sprays.

N.B.—Based on average tree crop of 325 fruits for Exp. I and 369 for Exp. II. Number of fruit harvested in Exp. I was estimated from a case count.

Overall drop was light and although the higher concentration 10 p.p.m. achieved some reduction on both orchards, differences were not significant. There was some indication that Bordeaux reduced the effect of 2,4-D.

1950 Trials

A further experiment was carried out in 1950 using 10 and 20 p.p.m. of the sodium salt of 2,4-D and 20 p.p.m. 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Spraying was done by knapsack on 28 May, the operator concentrating on wetting the fruits rather than giving a thorough foliage drench. Three hours after application, a drizzling rain shower occurred but by then the trees had dried.

TABLE II. EFFECT OF WATER SOLUTIONS OF 2,4-D AND 2,4,5-T ON PRE-HARVEST DROP OF WASHINGTON NAVEL ORANGES: 1950 TRIALS
(8 trees used for each treatment)

Treatment*	Av. No. fruit per tree	Av. No. sound fruit dropped per tree	Av. drop (%)	Reduction in drop (%)
Control: Water spray	284	41	15.3	—
10 p.p.m Sodium 2,4-D	350	31	8.7	42.9
20 p.p.m. Sodium 2,4-D	280	19	5.7	62.7
20 p.p.m. 2,4,5-T	300	10	3.3	78.6
Difference for significance 5%			2.8	
1%			2.1	

*Agral LN (3oz/100 gal) used with all sprays.

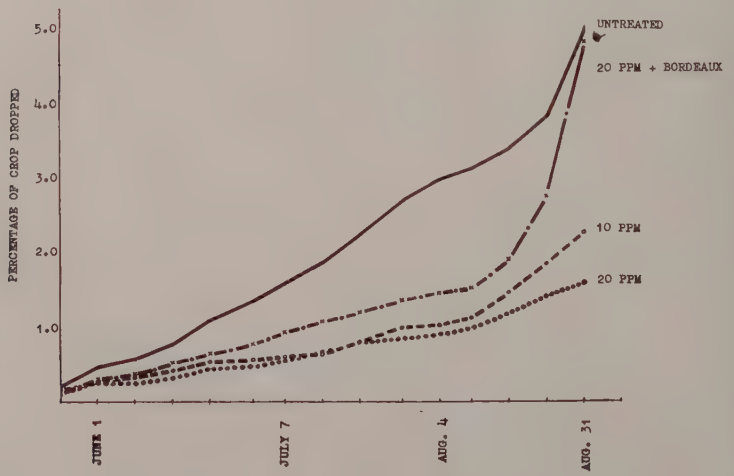

FIG. 1. Pre-harvest drop of Oranges with different treatments.

The 1949-50 summer was particularly dry and considerable fruit drop had occurred before spraying. Results are given in Table II. The first picking of fruit was made on 4 August.

Loss of sound fruit was reduced by both concentrations of sodium 2,4-D, but 20 p.p.m. gave a greater reduction than the lower concentration. The 20 p.p.m. 2,4,5-T spray was more effective than 20 p.p.m. sodium 2,4-D. Counts of dropped fruit at the end of the first week showed that the sprays had already taken effect. Further loss was reduced for a period of at least two months.

1951 Trials

In 1951 trees on two orchards were used. A range of concentrations and several salts of 2,4-D as well as 2,4,5-T and 2-methyl-4-chlorophenoxyacetic acid (MCPA) were tested. Mixtures of 2,4-D with Cuprox (a copper fungicide containing 30 per cent copper as copper oxychloride) and Bordeaux (3-4-50) were also included to determine the compatibility of 2,4-D with copper sprays used in orchards at that time of year. Details of sprays were as follows:

Experiment I

(1) Control—unsprayed
(2) 10 p.p.m. 2,4-D (as sodium salt)
(3) 15 p.p.m. 2,4-D (as sodium salt)
(4) 20 p.p.m. 2,4-D (as sodium salt)
(5) 20 p.p.m. 2,4-D (as sodium salt) + Cuprox (5 lb/100 gall)
(6) 20 p.p.m. 2.4-D (as sodium salt) + Bordeaux 3-4-50
(7) 20 p.p.m. 2,4-,5-T (as sodium salt)

Experiment II

(1) Control-unsprayed
(2) 20 p.p.m. Sodium 2,4-D
(3) 20 p.p.m. Triethanolamine 2,4-D (Formulation I)
(4) 20 p.p.m. Polyethylene glycol 2,4-D (Formulation II)
(5) 20 p.p.m. 2-methyl-4-chlorophenoxyacetic acid (as sodium salt) (Formulation III)

Sprays (3), (4) and (5) of Exp. II were prepared from formulations supplied by chemical firms. All hormone solutions were diluted to give the required equivalent concentration of the acid radicle. Before adding to the spray tank, sodium 2,4-D and 2,4,5-T were first dissolved in 50 per cent alcohol. The fungicide-hormone mixtures were prepared as for the 1949 trials.

Applications were made on 18 May using a standard portable sprayer equipped with two 4-nozzle brooms operating at 300 lb pressure. The weather at the time of spraying was bright and sunny. Little fruit drop had occurred from trees included in Exp. I when sprays were applied but some fruit had fallen from trees of Exp. II.

Harvesting commenced on Exp. I in the first week of September and for Exp. II on 23 August. Results are tabulated in Table III.

TABLE III. EFFECT OF WATER SOLUTIONS OF 2,4-D, 2,4,5-T AND MCPA ON PRE-HARVEST DROP OF WASHINGTON NAVEL ORANGES: 1951 TRIALS

Treatment*	Av. No. fruit per tree	Av. No. fruit dropped per tree	Av. drop (%)	Means of equiv. Angles	Reduction in drop (%)
EXPERIMENT I (6 trees used for each treatment)					
Untreated	798	40	4.9	12.7	—
10 p.p.m. 2,4-D (Sodium)	618	12	2.3	8.4	53.9
15 p.p.m. 2,4-D (Sodium)	578	8	1.5	7.0	69.4
20 p.p.m. 2,4-D (Sodium)	572	9	1.6	7.0	66.9
20 p.p.m. 2,4-D (Sodium) + Cuprox 5 lb/100	596	22	3.7	10.9	25.3
20 p.p.m. 2,4-D (Sodium) + Bordeaux 3-4-50	673	32	4.8	12.3	1.4
20 p.p.m. 2,4,5-T (Sodium)	712	10	1.6	7.0	67.3
Difference for significance 5%				1.9	
1%				2.5	

Treatment	Av. No. fruit per tree	Av. No. fruit dropped per tree	Av. drop (%)	Means of equiv. Angles	Reduction in drop (%)
EXPERIMENT II (5 trees used for each treatment)					
Untreated	368	39	10.7	19.1	—
20 p.p.m. 2,4-D (Sodium)	352	9	2.7	9.5	75.0
20 p.p.m. 2,4-D (Triethanolamine-Formulation I)	356	7	2.1	8.0	80.5
20 p.p.m. 2,4-D (Polyglycolester-Formulation II)	361	8	2.3	8.6	78.6
20 p.p.m. 2-methyl-4-chlorophenoxy-acetic acid (Formulation III)	362	20	5.5	13.3	48.8
Difference for significance 5%				2.7	
1%				3.8	

*Agral LN (2½ oz/100 gal) used with all sprays.

Sprays of 10, 15 and 20 p.p.m. 2,4-D were effective in reducing drop of sound fruit over the total period of the experiment.

All 2,4-D salts tested and sodium 2,4,5-T gave comparable control of drop, while MCPA gave inferior control. A similar number of fruit was lost from control trees of both blocks and although the percentage drop differed markedly because of total crop differences, the degree of saving achieved by treatment was similar.

The cumulative drop of sound fruit from Treatments (1), (2), (4) and (6) of Exp. I is represented graphically in Fig. 1.

While data in Table III show that by harvest there was little decrease in drop from the Bordeaux-2,4-D treatment, it is clear from Fig. I that Bordeaux shortened the duration of effectiveness of the hormone rather than nullified its effects completely. The mixture reduced drop for a period of only thirteen weeks although less effectively than 2,4-D used alone. After thirteen weeks, the rate of drop increased rapidly so that at the end of the experiment as many sound fruit had dropped from treated trees as from controls. A similar effect was apparent with the Cuprox-2,4-D treatment.

Foliage and Fruit Effects

When sprays were applied each season, late growth had ceased on most trees but some young growth was present on a few treated trees. No foliage effects were observed on any of these trees, however, and the younger shoots must have hardened sufficiently to resist injury.

Examination of harvested fruit disclosed no effect of treatment on fruit quality.

DISCUSSION

These experiments confirm for New Zealand conditions the results of overseas workers that dilute sprays of 2,4-D and similar chemicals will reduce pre-harvest drop of Washington Navel oranges and effect an economic saving of sound fruit.

The concentrations of 2,4-D found to give good control in the above trials are similar to those used in Australia (Frith, 1950; Jones, 1951), but are higher than the recommended strength for Californian orchards (Stewart, 1948). It is to be noted, however, that oil based ester forms of 2,4-D, frequently used in California, are recognised to be slightly more effective than the water soluble salts used in the above experiments. Another factor influencing the use of lower concentrations may be a more extended season of active growth and consequent greater risk of tree injury. While both 2,4-D and 2,4,5-T at higher concentrations gave satisfactory control of drop in the above experiments, 2,4,5-T gave no consistently better control than 2,4-D. Thus, at present, 2,4-D seems the chemical most suitable for general use (Davison and Cederman, 1951, 1952) because of its cheapness and availability in the form of hormone weedkiller preparations.

Such sprays are effective if applied either before or after fruit drop has commenced.

For practical purposes a single spray applied in late autumn will reduce fruit loss for periods of at least fifteen weeks up to the time of harvesting in August or September. Although the above experiments

have been confined to Washington Navel oranges in Kerikeri, 2,4-D sprays can be expected to give similar results in other New Zealand citrus districts and on other citrus varieties which are susceptible to pre-harvest drop.

The results obtained with copper sprays do not agree with those of Stewart, Klotz and Hield (1947) who state, without giving details, that 2,4-D can be safely mixed with a range of sprays including Bordeaux. Frith (1950) showed that, over a period of approximately nine weeks, the effectiveness of 2,4-D applied with Bordeaux and white oil was not reduced. These latter results, however, do not contradict the results of the experiments reported in this paper, since the control ceased after thirteen weeks, and figures prior to this show a significant reduction of drop. The reason for this partial incompatibility of 2,4-D with both Bordeaux and Cuprox in the above trials is not clear. A similar effect has been reported (Kadow and Hopperstead, 1941) when *a*-naphthaleneacetic acid, used for controlling pre-harvest drop of apples, was mixed with sprays containing lime.

As no foliage injury was observed on any experimental block at Kerikeri for three successive years, it is evident that leaf curling can be avoided by ensuring that strengths above 20 p.p.m. are not used and by delaying applications until trees have ceased growth.

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RADIATION INJURY FROM RADIOACTIVE PHOSPHORUS IN TRACER EXPERIMENTS WITH PLANTS

By K. Strzemienski, Soil Bureau, Department of Scientific and Industrial Research, Wellington

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Summary

An experiment was made with short-rotation ryegrass grown in pots with four levels of radioactivity, viz. 40, 160, 640, and 2,560 μc per gram of phosphorus. No significant differences have been found in either dry matter production or phosphate absorption and distribution. These results and those of other workers are discussed.

Introduction

In any experiment using a tracer element an obvious condition for the results to be valid is that the processes studied are not influenced by the tracer isotope; if they are, results obtained are not generally true, but apply only in the presence of the tracer element. It has long been known that radiations from radioactive elements may influence living organisms, but Hendricks and Dean (1948 a, b) reported pot experiments with perennial ryegrass where radioactive phosphorus in amounts as large as 625 microcuries (μc) per kilogram of soil did not influence the yield either way. This experiment was generally accepted by workers interested in fertilizer studies as a sufficient proof that the radioactive-phosphorous tracer does not affect the vital processes of plants and can be safely used; the more so, as it is needed in such fertilizer experiments in amounts much smaller than those applied by Hendricks and Dean. Scott Russell and Martin (1949), however, demonstrated serious radiation damage in barley grown for 6 days in solution cultures containing radioactive phosphate, even at a concentration of 10 μc per litre, and noticed that phosphate absorption was affected to a greater extent than dry matter production. Scott Russell, Adams and Martin (1949b) described pot experiments with barley and wheat where considerable changes, especially in the ratio of fertilizer phosphorus to soil phosphorus taken up by the plants, were caused by radioactive phosphorus present at levels of concentration well below those commonly used in such experiments.

Since several pot experiments on phosphate uptake had been made in this laboratory with the use of radioactive tracer, it was felt necessary to check on a possible influence of radiation on the behaviour of plants in conditions similar to those of the previous experiments in this laboratory.

Experimental Methods

Ordinary 5-in. earthenware pots, coated inside and outside with a vinyl chloride acetate resin were brought to equal weight by placing paraffin-waxed gravel at the bottom of each pot, and were filled with a dark grey topsoil from a Kaitoke silt loam at Silverstream, near Wellington. This soil derived from a greywacke sand-

stone was known from a previous pot experiment to be very poor in available phosphate. Chemical analysis of this soil before any treatment gave the following data:

Citric acid soluble P_2O_5, 0.006%; pH, 5.1; C, 4.2%; N, 0.24%; C/N, 18; base exchange capacity, 16.0 m.e./100g; total exchangeable bases, 4.6 m.e./100g; exchangeable Ca, 3.4 m.e./110g; Mg, 1.2 m.e./100g; K, 0.48 m.e./100g.

A basal dressing containing all nutrient elements except phosphorus, and calcium carbonate were added to the soil in bulk, well before potting, and thoroughly mixed repeatedly. The pH of the soil was 7.0 at the time of seeding. Each pot was filled with 700g of the soil, equal to 579g soil on a dry-matter basis. The pots were sown each with 19 seeds of short-rotation ryegrass on 25 October, 1950, and later thinned so that from 11 to 16 plants remained in each pot, the number of plants being the same in all pots within the block. Each block comprising six pots, each of a different treatment, were kept in wooden racks in a glasshouse, and were shifted and rotated by half a turn every day. There were six replicates provided for each treatment. On 7 November, 1950, 25 ml of phosphate solution followed by 15 ml of water was applied per pot. This solution contained 30 mg P_2O_5 in the form of monopotassium phosphate. The no-P treatment (I) received a corresponding amount of water. Treatment II received only inert phosphate, and Treatments III—VI received increasing amounts of P^{32} in the ratio 1 : 4 : 16 : 64.

The radioactive material was obtained from the A.E.R.E., Harwell, England, as a carrier-free separated isotope.

The plants developed normally. The differences between the no-phosphate plants and the others became unmistakable from 15 November, i.e. 8 days after phosphate application. No differences could be observed between the other treatments. The top parts were collected on 13 December, 1950, i.e. 36 days after phosphate application, and the roots on following days.

The whole crop from each pot was treated as a sample. After preliminary drying in a forced draft oven at 56°C, the samples were dried at 103°C, and weighed. After wet combustion with a mixture of perchloric and nitric acids, the total phosphorus content was determined by the gravimetric method of Lorenz (1901)—a "centigram procedure"—as modified by Neubauer and Lücker (1912), but using sintered glass crucibles for filtration and weighing. The ammonium phosphomolybdate thus obtained was dissolved in ammonia, standard phosphate solution added to make up to the amount of P_2O_5 contained in the standard for radioactivity measurements, and the ammonium-magnesium phosphate precipitated and filtered through sintered glass crucibles specially designed for this purpose. Radioactivity measurements were made with Geiger-Müller counters by comparison with the standard. Aliquots of the fertilizer solution actually used for the pot experiment were treated similarly to the plant samples and served as standards in the radioactivity measurements. These methods will be described elsewhere in detail.

Results shown in Table I are averages of six replicates for each treatment. Figures obtained from one pot of Treatment II were not included in the calculations, because this pot contained only seven plants. Amounts calculated by means of the Yates-Wishart "missing-pot formula" were used instead.

RESULTS AND DISCUSSION

Treatment I (see Table I), by yields equal to roughly one sixth or one seventh of the yields of other treatments, demonstrates the deficiency of this soil in available phosphate. This also explains why at least two thirds of the total phosphorus taken up by the plants in Treatments III—VI came from the fertilizer, and at most only one third from the soil. Otherwise, this treatment is of no great importance for the purpose of this experiment. It has not been included in the analysis of variance.

Differences in yields and in uptake of phosphate between the other treatments are very small and are not statistically significant at the 5 per cent level. The same applies to the various ratios calculated and shown in Table I. This is a rather reassuring result, the more so as there is reason to believe that exposure to radiation was more severe than in the writer's previous experiments: the short-rotation ryegrass develops faster than perennial ryegrass used previously; the radioactive phosphate was applied here to already quickly developing plants while in the older experiments it was in most cases applied before seeding. Moreover, amounts of P^{32} used in this experiment were higher than those used before.

There are, however, some points that warn against too much optimism. The ratio, $\frac{\text{fertilizer } P_2O_5 \text{ in tops}}{\text{fertilizer } P_2O_5 \text{ in roots}}$, shows a slight but distinct tendency to decrease as the radioactivity increases. Though on analysis of variance alone, the differences are far below 5 per cent level of significance, the calculated coefficient of regression fairly closely approaches this level, being 2.013 actually as compared with 2.074 necessary. This has not been caused by a haphazard combination of the two components; the fertilizer P_2O_5 drops slightly from the lowest radioactivity level—Treatment III—to the higher ones, though it is practically equal for Treatments IV, V and VI, in which differences have to be regarded as fortuitous. However, the rise in the fertilizer phosphate in the roots is more noticeable with increasing radioactivity. A similar trend appears in the ratio of total phosphorus in tops to total phosphorus in roots in Treatments III—VI, but Treatment II is entirely out of line with a figure below that of the highest radioactivity level (Treatment VI). Since a simple explanation for this result would be difficult to give, the series of ratios appears very likely to be due to chance. Consequently, the doubt may arise about the validity of the apparent trend in the ratios of the fertilizer phosporus in tops to that in roots which could be suspected of looking uniform only because of the absence of the no-radioactivity treatment for which the necessary data could not, of course, be determined. Since the percentage of fertilizer phosphate on total phosphate

basis is fairly uniform in the radioactive series for the tops and roots respectively, averages of these data were used to calculate the hypothetical amounts of fertilizer phosphate present in the shoots and roots of the plants grown on inert phosphate (Treatment II). The figure thus calculated is again out of line, being almost identical with that for the highest radioactivity level. (The relevant data are shown in brackets in Table I). Even if the highest percentage—80.4 from Treatment IV—is used for the tops and the lowest—67.1 from Treatment II—for the roots, the figure of 2.08 so obtained is still out of the general line, being fairly close to the

TABLE I. EFFECT OF VARYING QUANTITIES OF PHOSPHORUS-32 ON RYEGRASS

Treatment No.	I	II	III	IV	V	VI
TREATMENTS						
P_2O_5 added (mg/pot)	0	30	30	30	30	30
P^{32} added (μc/pot)	0	0	0.5	2.1	8.4	33.5
P^{32} μc/g P_2O_5	0	0	17.5	69.8	279	1117
P^{32} μc/g P	0	0	40	160	640	2560
P^{32} μc/kg soil (d.m.)	0	0	0.90	3.6	14.5	58
RESULTS						
Tops						
Yield, d.m. (g/pot)	0.32	2.15	2.26	2.14	2.21	2.17
P_2O_5 total (mg/pot)		6.52	6.85	6.24	6.53	6.50
P_2O_5 from fertilizer (mg/pot)		(5.02)*	5.31	5.02	4.90	4.88
Fertilizer P_2O_5 as % total		(77.0)*	77.6	80.4	75.0	75.0
Roots						
Yield, d.m. (g/pot)	0.25	1.27	1.28	1.21	1.28	1.26
P_2O_5 total (mg/pot)		3.75	3.22	3.15	3.49	3.52
P_2O_5 from fertilizer (mg/pot)		(2.61)*	2.16	2.23	2.41	2.51
Fertilizer P_2O_5 as % total		(69.5)*	67.1	70.6	69.0	71.4
Whole Plants						
Yield, d.m. (g/pot)	0.57	3.42	3.54	3.35	3.49	3.43
P_2O_5 total (mg/pot)		10.27	10.06	9.39	10.02	10.02
P_2O_5 from fertilizer (mg/pot)			7.47	7.25	7.30	7.39
P_2O_5 from soil (mg/pot)			2.59	2.15	2.72	2.63
Fertilizer P_2O_5 as % total			74.2	77.1	72.9	73.8
Ratios						
$\frac{\text{Yield of tops}}{\text{Yield of roots}}$	1.27	1.69	1.76	1.77	1.72	1.72
$\frac{\text{Total } P_2O_5 \text{ in tops}}{\text{Total } P_2O_5 \text{ in roots}}$		1.74	2.13	1.98	1.87	1.85
$\frac{\text{Fertilizer } P_2O_5 \text{ in tops}}{\text{Fertilizer } P_2O_5 \text{ in roots}}$		(1.92)*	2.46	2.25	2.03	1.94

* These figures are purely conjectural, calculated on the assumption that the fertilizer P_2O_5 in Treatment II plants made a percentage of Total P_2O_5 equal to the average of Treatments III - VI.

ratio for Treatment V. These are obviously mere speculations, as no direct information can be obtained of the actual percentage of the fertilizer phosphate in Treatment II, but they give some support to the assumption that trends in both ratios, viz. of total and of fertilizer phosphorus, are purely accidental. These ratios still appear to deserve some closer study in future experiments of this kind.

Papers so far published on radiation damage from P^{32} often reported very different results, so that even for solution cultures it does not appear permissible to set a general safety level. In some cases, lower levels of radioactivity affected the phosphate uptake while higher levels were needed to affect yield; in other cases the reverse was true, as a comparison of experiments reported by Blume *et al.* (1950) with those of Scott Russell *et al.* (1949a, 1950a, b.) shows. Similarly, sometimes shoots, sometimes roots are more affected. Still less predictable are results obtained from experiments with plants grown in soils. So Bould *et al.* (1951), when experimenting with one soil, obtained significant differences in dry weights and phosphate uptake at some radioactivity levels (much higher ones than those used by Scott Russell *et al.* (1949b, 1950a)), but with another soil—presumably using the same technique—no influence of the radioactivity on the plants was found.

From the results already known, one conclusion can be safely drawn: that some yet unknown factors must interfere. An example showing the complexity of the problem is seen in Table II where data are reproduced from an experiment with barley by Scott Russell *et al.* (1949b, 1950a). The authors interpreted these results by assuming that the fertilizer applied as a top-dressing in solution was retained by the upper 2 in. of the soil, and that the roots were injured in that layer and consequently absorbed the less of the fertilizer phosphate the larger was the injury. This seems a reasonable explanation for the data shown in the upper part of Table II. If, however, the soil phosphate and the total phosphate absorbed by the plants are calculated from the data given, a considerable increase in soil phosphate absorption with increasing radioactivity appears. For this occurrence, injury to roots in the upper layer is of itself no longer a sufficient and obvious explanation.

Another reason for extreme caution in generalizations is the comparatively low levels of radioactivity at which microscopically discoverable histological and cytological disorders may appear without necessarily being associated with any measurable deviations in either dry matter production or phosphate absorption and distribution. (Ehrenberg *et al.*, 1949; Arnason *et al.*, 1948; Blume *et al.*, 1950). Moreover, considerable differences in susceptibility even between closely related plant species have been found.

The safest procedure to adopt in tracer experiments on phosphate uptake seems to be, for the time being, to include at least three levels of radioactivity. Two will be sufficient only if there is no difference between them in the quantities studied. This means of course a serious complication in carrying out such investigations.

Conclusions that may justifiably be made are:

(1) In the experiment described above P^{32} applied in concentra-

tions from 40 to 2,560 μc per gram of P either did not influence results at all, or only to a negligible extent.

(2) Though it is still for the future to develop sound foundations for inferring from one experiment on radiation injury to another, it does not appear very probable that in previous experiments made in this laboratory in similar conditions but with lower radioactivities, the results were seriously distorted by the influence of radiation.

TABLE II. * EFFECT OF VARYING ACTIVITIES OF PHOSPHORUS-32 ON GROWTH OF BARLEY

Treatment					
μc P^{32}/culture pot	0	0.16	0.8	4.0	L.S.D.
μc P^{32}/ g P	0	18	92	460	
Dry weight (g/pot)					
Shoots	2.38	2.46	2.51	2.63	-
Roots	1.23	1.22	1.26	1.17	-
Fertilizer Phosphate Absorbed (g/pot)		3.42	2.75	2.45	0.79 (5%)
Fert. Phosphate Absorbed / Soil Phosphate Absorbed (%)		16.74	12.4	7.9	

* The above Table is reproduced from Scott Russell et al. (1949c). From their data, the following figures have been calculated:

Soil Phosphate Absorbed (mg/pot)	20.43	22.18	31.01
Total (i.e. Soil plus fertilizer) Phosphate Absorbed (mg/pot)	23.85	24.93	33.46

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OCCURRENCE OF LUCERNE-MOSAIC VIRUS IN NEW ZEALAND

By P. R. Fry, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

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Summary

Virus symptoms were found on white and red clover plants growing in trial plots at Palmerston North and on lucerne plants at Lincoln. From symptoms, physical properties and cross protection tests, a virus recovered from these plants was identified as lucerne-mosaic virus. Incidence of infection in pastures is not known. Three strains of the virus were recovered from infected clover plants.

Introduction

Virus symptoms were found on a high percentage of plants in plots of selected clover lines at Palmerston North in January, 1950. Two viruses, lucerne-mosaic and pea-mosaic, were found to be present. This paper records the identification of lucerne-mosaic virus which has not previously been found in New Zealand.

Incidence

A number of clover plants collected from an experimental plot at Palmerston North were tested for infection with lucerne-mosaic virus by inoculation to tobacco (*Nicotiana tabacum* L.). The following numbers of plants were infected: 24 out of 24 white clover (*Trifolium repens* L.), 1 out of 17 red clover (*T. pratense* L.), and the only strawberry clover (*T. fragarifolium* L.) plant tested. Twelve subterranean clover (*T. subterraneum* L.) plants were free from infection.

A further record of the disease was made in an experimental plot of lucerne at Lincoln.

Four pastures in the Palmerston North District were inspected in January, 1951, and the following information obtained: (1) a ten-month old pasture four hundred yards from the infected plot showed no virus symptoms; (2) a three-year-old pasture adjoining the infected plot showed symptoms on red but not white clovers; (3) a ten-month-old pasture five miles from the infected area showed no virus symptoms; and (4) a four-year-old pasture adjoining (3) was also free from symptoms.

No lucerne-mosaic virus was recovered from any of the 57 red and white clover plants collected in approximately equal numbers from the above pastures. The symptoms on red clovers in (2) were caused by pea-mosaic virus.

Symptoms

Naturally Infected Hosts

White Clover: Symptoms on white clover plants are variable. Plants may show no obvious symptoms or develop pale chlorotic spots on young leaves followed by diffuse mottling as leaves age. Necrotic spotting or etching along the veins sometimes occurs and is often accompanied by interveinal yellowing. Plants which do not develop leaf symptoms usually grow vigorously, while those with mottled or necrotic leaves remain stunted.

Lucerne (*Medicago sativa* L.): Infected plants are usually stunted with short intermodes and small leaves which may be curled or twisted. Leaves may either be mottled with pale green or yellowish areas or develop yellow ringspots (Fig. 1).

Strawberry Clover: The only infected strawberry clover plant so far found showed no symptoms.

Red Clover: As naturally infected specimens have also been infected with pea-mosaic, the field symptom of lucerne-mosaic is not known.

B. Experimental Hosts

Symptoms described are those produced by virus recovered from a white clover plant and cultured in tobacco or *Nicotiana glutinosa* L. This culture is referred to later as Strain A.

FIG. 1.—Lucerne-mosaic virus on naturally infected lucerne. Leaves showing mottling and ringspotting.
Nat. size. (*Photo by S. A. Rumsey*)

FIG. 2.—Strains of lucerne-mosaic virus on tobacco. Leaves inoculated with, right to left, strains A, B, and C, showing local lesions seven days after inoculation.
x 1/5. (*Photo by S. A. Rumsey*)

FIG. 3.—Leaves from same plants as Fig. 2 showing systemic infection.
x 1/4. (*Photo by S. A. Rumsey*)

FIG. 4.—Lucerne-mosaic virus on tobacco. Leaves of tobacco seven days after infection by aphids, showing concentric lesions and etched lines following the veins.
x 1/2. (*Photo by S. A. Rumsey*)

Tobacco: Pale green or white etched ringspot local lesions appear on leaves from three to six days after inoculation. The lesions spread to form necrotic etched markings bordering the veins (Figs. 2 and 4).

Vein clearing of young leaves appears after about five or seven days and soon gives place to pale green etching of the vein network, often accompanied by chlorotic mottling (Fig. 3) and necrotic spotting. Symptom intensity is greatest on the first leaves to show systemic infection, those developing later often being symptomless.

Nicotiana glutinosa: Dark brown necrotic lesions or ringspots appear on inoculated leaves after four to six days. Systemic symptoms appear six to ten days after inoculation as vein-clearing and white necrotic etching along the veins. The first young leaves to show symptoms soon develop a severe general necrosis which often occurs only on one side of the midrib but may kill the entire leaf. Symptoms on later formed leaves are usually less severe, consisting of whitish vein etching or mild mottling.

In both *N. glutinosa* and tobacco, symptoms are generally mild in summer and severe in winter.

Cucumber (*Cucumis sativus* L.): Ten to fourteen days after inoculation yellowing of fine veins appears in small isolated areas. Fully developed symptoms consist of chlorotic spotting, vein yellowing and yellowing of small areas of leaf (Fig. 5). Leaves are often distorted due to retarded growth in the yellowed areas.

French Bean (*Phaseolus vulgaris* L.): About seven days after inoculation, reddish-brown necrotic local lesions (Fig. 6) appear as pin-point spots which slowly enlarge and may reach 5mm diameter. There is no systemic spread, infection remaining localized in the lesions. In winter lesions are produced in considerable numbers, but in summer few, if any, appear.

Fig. 5.—Lucerne-mosaic virus on cucumber. Leaves of Short Prickly cucumber one month after plants were inoculated, showing chlorotic spotting, yellowing of veins and small areas.
x 1/2. (*Photo by S. A. Rumsey*)

Pea (*Pisum sativum* L.): Necrotic local lesions appear within seven days of inoculation and inoculated leaves soon wither and fall. Necrotic spotting also occurs regularly on the first two or three young leaves. Leaves developed later are often symptomless but may develop necrotic spots. Infected plants are usually stunted.

Broadbean (*Vicia faba* L.): (Fig. 7): Necrotic lesions are produced on inoculated leaves after five to seven days. Systemic infection causes necrotic spots on young leaves together with vein necrosis followed by collapse of the affected areas. Necrotic streaks later appear in the stem, causing wilting and collapse. Lateral shoots may develop beneath the necrosed area of stem and grow normally for a time before they, in turn, become necrotic.

FIG. 6.—Lucerne-mosaic virus on French bean. Leaf of Canadian Wonder bean eight days after inoculation, showing necrotic local lesions.
x 2/5. (*Photo by S. A. Rumsey*)

FIG. 7.—Lucerne-mosaic virus on broadbean. Broadbean plants nine days after inoculation, showing necrotic lesions on lower leaves and systemic necrosis.
x 5/12. (*Photo by S. A. Rumsey*)

Subterranean Clover, var. Mount Barker: Vein clearing and necrotic etched lines adjacent to the veins develop seven or eight days after inoculation. About a month after infection new leaves show mild mottling without necrosis. There is no apparent reduction in vigour.

Red Clover, var. Congress: Two weeks after inoculation veins of young leaves become necrotic and the leaves are curled and twisted. Infected plants are stunted.

METHODS OF TRANSMISSION

Mechanical

The virus is readily transmitted to susceptible species by leaf rubbing with infective sap using fine carborundum powder.

Aphid

Weimer (1934) demonstrated that the pea aphid *Macrosiphum pisi* Kltb. could transmit lucerne-mosaic virus. Porter (1935) used

M. euphorbiae Thomas in transmission studies with the potato calico strain, and Kovacevski (1942) transmitted the chilli strain with *Myzus persicae* Sulz.

Trials were carried out with *M. persicae* as vector, clover plants as source of infection, and White Burley tobacco plants as indicators. Aphids were prefasted for from four to twenty-seven hours, fed for five to ten minutes on infector plants and left on the indicators for several hours. Three to eight aphids were used in each test. Eleven of the twenty-two inoculated plants became infected. Symptoms followed the same course as in mechanically inoculated plants. Concentric ringspot lesions (Fig. 4) appeared where aphids had fed, and systemic spread developed as described earlier.

A further trial was carried out using subterranean clovers as indicator plants. Aphids were prefasted overnight and fed for five minutes on infected white clover leaves before six were placed on each indicator plant. Ten out of twelve inoculated plants became infected.

Physical Properties

Thermal Inactivation

Pierce (1943) and Zaumeyer and Wade (1935) found that lucerne-mosaic virus was inactivated by exposure for ten minutes to temperatures above 63°C. Zaumeyer (1938) later reported strains with inactivation points between 65° and 70°C and 70° and 75°C.

Sap extracted from both White Burley tobacco and white clover plants was inactivated after ten minutes treatment at 65°C.

Aging in vitro

The longevity in expressed sap was given by Pierce (1934) as seven to nine days and by Zaumeyer (1938) as four to five days.

Sap from infected *N. glutinosa* plants stored at room temperature remained infective for four but not five days.

Tolerance to Dilution

Pierce (1943) recorded a dilution end point of 1 in 1,000, and Zaumeyer (1938) found the end point of his type virus was 1 in 2,000, while that of other strains was between 1 in 3,000 and 1 in 5,000. Ross (1941) showed that the concentration of lucerne-mosaic virus in tobacco plants fell off rapidly with time from a maximum approximately twelve days after inoculation. The host used as source of infection also influences the concentration of virus in the inoculum. This was shown in the present trials when end points of 1 in 1,000, 1 in 10,000 and 1 in 100,000 were obtained with sap from tobacco and *N. glutinosa* plants which had been infected for fourteen days, and from naturally infected white clover.

Identity

Cross protection tests were carried out by inoculating with the virus under study, White Burley tobacco plants, systemically infected with cucumber-mosaic virus, potato viruses X and Y and tobacco-mosaic virus. In all cases numerous ringspot local lesions were produced, indicating that this virus is not related to any of the other four.

Symptoms on lucerne are similar to those described for lucerne-mosaic virus by Weimer (1934). Reaction of tobacco, bean, broad-bean and cucumber are the same as described by Zaumeyer (1938). Physical properties are in agreement with those given by Pierce (1934), Zaumeyer (1938), and Zaumeyer and Wade (1935).

The virus described in this paper is therefore closely related to those studied by the above authors and is identified as lucerne-mosaic virus (Imp. Mycol. Inst., 1946).

Strains

Several strains of lucerne-mosaic virus have been reported on various hosts. Zaumeyer (1938) differentiated three strains occurring on lucerne. Black and Price (1940) identified the virus causing potato calico as a strain of this virus. Kovacevski (1942) recorded a strain on chilli in Bulgaria, and Berkeley (1947) another on pepper in the U.S.A. Kreitlow and Price (1949) found a further strain on Ladino clover plants.

As the author did not have access to these virus isolates, no direct comparisons with the strains occurring in New Zealand could be made. The following evidence is presented to show that at least three strains of lucerne-mosaic virus have been recovered from plants in a small plot of clover. Table I gives a summary of symptoms produced by inoculation with isolates of the virus from three different clover plants.

TABLE I

STRAINS OF LUCERNE-MOSAIC VIRUS.

SYMPTOMS CAUSED BY VIRUS ISOLATES FROM THREE CLOVER PLANTS

Source of virus	Tobacco	French bean	Broad bean	White clover	Subterranean Clover		Red Clover	
					Mt. Barker	Tallarook	Broad Red	Montgomery
A. White clover	Etched ring-spot local lesions, systemic etching and mottle	Necrotic local lesions only	Necrotic local lesions, systemic necrosis	Mottle	Vein clearing, etching, mild mottle	No infection	Vein necrosis, leaf distortion, stunting	No infection
B. White clover	"	"	"	"	"	Necrotic etching, stunting and death	"	Vein necrosis, leaf distortion, stunting
C. Strawberry clover	Chlorotic lesions, mild mottle	Vein necrosis spreading to petioles and stems	"	Symptomless carrier	"	"	Transient vein clearing	

From this table it may be seen that while Strain B caused severe diseases on Tallarook subterranean clover and Montgomery red clover, Strain A failed to infect either of these varieties. Strain C caused the same symptoms as A and B on broad bean and Mount Barker subterranean clover, and the same as B on Tallarook subterranean clover. Reaction of all other hosts to Strain C was different from that to A or B. This suggests that Strain A and B are more closely related than either A and C or B and C.

A similar result was obtained in cross-protection tests carried out on White Burley tobacco plants. Infection with Strain A protected against Strain B and Strain B protected against A. Neither A nor B protected against C but plants infected with C were protected against infection with A or B.

Economic Importance

The extent to which this virus occurs in pastures in New Zealand is not known, but as it causes stunting of white clover and lucerne it must reduce yields wherever these plants are infected. Eradication of this disease from pastures would be difficult since the virus is spread by aphids, and undetected sources of infection could exist in some clover varieties which may not show obvious leaf symptoms. Breeding for resistance is likely to be difficult because the virus occurs in a number of strains.

Acknowledgment

The author wishes to thank Mr. L. Corkill, Grasslands Division, Department of Scientific and Industrial Research, for making field inspections and collecting plants.

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SCREENING TRIALS OF ACARICIDES FOR CONTROL OF TOMATO RUSSET MITE (*Phyllocoptes Lycopersici* Masee) IN THE GLASSHOUSE

By K. P. Lamb and H. Jacks, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 21 March 1952)

Summary

Six therapeutant trials were carried out under glasshouse conditions on tomato plants infested with *Phyllocoptes lycopersici* Mass.

Contact effect of treatments was assessed by determination of relative survival of mites 24 hours after treatment.

The following materials were effective: Arathane, 0.2 per cent spray; micronized sulphur dust, at 2.5 and 5.0 g. per plant; EPN-300 0.1 per cent spray; Aldrin, 0.1 per cent spray; Midol T.10, 1 per cent spray; Midol 100 + oil, 1 per cent spray; Midol 100, 1 per cent spray; Folidol, 0.1 per cent spray; Nicotine sulphate, 0.17 per cent spray; Aramite, 0.15 per cent spray; Summer oil, 1 per cent spray; and Pestox III, 0.05 and 0.1 per cent sprays.

Higam, 0.025 and 0.1 per cent sprays, Dieldrin, 0.1 per cent spray, and Marlate, 0.1 per cent spray were of doubtful efficacy.

DDTemul, 0.1 per cent spray, Neotran, 0.1 per cent spray, and PPC, 0.1 per cent spray were ineffective in that they failed to reduce the population on treated plants significantly below those on the controls.

Introduction

The tomato russet mite (*Phyllocoptes lycopersici* Massee) was first recorded in New Zealand by Cottier and Taylor (1937), and it has since become an increasingly serious pest of both indoor and outdoor tomato crops. This paper reports the results of a number of screening trials of newer acaricides against this mite.

Acaricide Materials

Sulphur dust, reduced by a micronizer reduction mill to particle size of 2-5 μ.

Nicotine sulphate (40 per cent nicotine), a water miscible preparation.

Summer oil, a petroleum oil emulsion of viscosity 80 (Redwoods I at 100° F.) and unsulphonated residue of 95 per cent.

Pestox III (66 per cent bis-dimethylamino phosphonous anhydride plus a wetting agent), a water miscible preparation.

Arathane (25 per cent dinitro capryl phenyl crotonate), a wettable powder.

Aramite (15 per cent β-chloro ethyl β- (p-tertiary-butyl phenoxy) α-methylethyl sulphite) a wettable powder.

Folidol (22.4 per cent parathion (diethyl p-nitro-phenyl thiophosphate)), an emulsifiable preparation.

EPN-300 (27 per cent ethyl *p*-nitrophenyl thionobenzene phosphonate), an emulsifiable preparation.

Aldrin (23 per cent 1, 2, 3, 4, 10, 10 - hexachloro - 1, 4, 4a, 5, 8, 8a, hexahydro - 1, 4, 5, 8, - dimethanonaphthalene), an emulsifiable preparation.

Dieldrin (24 per cent 1, 2, 3, 4, 10, 10 - hexachloro - 6, 7, - epoxy - 1, 4, 4a, 5, 6, 7, 8, 8a - octahydro - 1, 4, 5, 8 - dimethanonaphthalene), an emulsifiable preparation.

Higam (25 per cent lindane (99 per cent pure γ BHC)), a wettable powder.

Neotran (40 per cent bis (*p*-chlorophenoxy) methane), a wettable powder.

Marlate (44 per cent methoxychlor (50 per cent dimethoxydiphenyl trichloroethane)), a wettable powder.

DDTemul (20 per cent *p,p,* i. DDT), an emulsifiable preparation.

Midol T. 10, an emulsifiable preparation containing 2.7 per cent BHC (0.7 per cent γ isomer), plus 4 per cent azobenzene, plus 0.3 per cent parathion.

Midol 100 + oil, an emulsifiable preparation containing 4 per cent azobenzene, 0.4 per cent rotenone, 2 per cent pyrethrins and 5 per cent mineral oil.

Midol 100, an emulsifiable preparation containing the same ingredients as above except for mineral oil.

P.P.C. (50 per cent chlordane (1, 2, 4, 5, 6, 7, 8, 8 - octochloro - 4, 7 methano - 3a, 4, 7, 7a tetra-hydro-indane with related compounds)), an emulsifiable preparation.

All spray materials were diluted with water before application.

Experimental Methods

Tomato plants growing in 8-in. pots were infested when young with *Phyllocoptes lycopersici*. When heavily infested the plants were taken for treatment. All treatments were replicated, each replication consisting of one plant. In each trial check plants were sprayed with water. Mite populations were determined on selected leaves from each plant before treatment and 24 hours after treatment and the acaricides assessed according to the relative number of survivors present after treatment. All counts were subjected to statistical analysis.

In Trial I, three replications were employed for each treatment, and before treatment the mites present on four leaflets per plant were counted and the areas of the leaflets measured. There was no significant correlation between leaf area and number of mites present. Sprays were applied with a hand atomizer at a rate of 228 ml per plant and dusts with a plunger type, hand operated dust gun at 2.5g. or 5g. per plant. Twenty-four hours

after application, four further leaflets were sampled from each plant and counts made of live and dead mites. Counts on a further four leaves were taken 7 days and 14 days after treatment.

In Trial II, four replications were taken for each treatment and the mites counted on three leaflets per plant. Materials were applied as in Trial I and at the same rate per plant. Twenty-four hours after treatment the mites which survived were counted on a further three leaflets per plant. In this and subsequent trials only live mites were counted as these counts were found more reliable than counts of dead mites.

In Trials III, IV, V and VI, four replications were employed and a single leaflet was taken from each plant for mite counts. In Trial V, sprays were applied by a hand atomizer as before, but in Trials III, IV and VI, a different method of spray application was employed. Here plants were placed on a rotating table and sprays were applied, from a small power sprayer, at a rate of 300 ml per plant at 35 lb pressure.

Prior to analysis all counts were transformed to log $(n + 1)$. Differences between the mean transformed counts before and after treatment were taken to compensate for initial variation in numbers of mites between treatments. These differences were taken as bases for comparison of the 24-hour contact effect of treatments. Where counts were made 7 days and 14 days after treatment these were corrected for variation due to differential survival after treatment by subtracting the 24-hour transformed means from the transformed means of the later counts. This provided a comparison of the rates of population increase after treatment, irrespective of mortality in the first 24 hours.

Results of these trials are summarized in Tables I—VI.

Table I. Table of Means, Spray Trial I.

Treatment	Mean no. live mites per sample				Differences between means of transformed data		
	Before treatment	24 hr after treatment	7 days after treatment	14 days after treatment	24 hr after	7 days after	14 days after
1. Check	356	1213	792	721	—0.58	—0.29	—0.26
2. Sulphur dust: 2.5g/plant	269	10	39	11	1.18	0.27	—0.22
3. Sulphur dust: 5g/plant	820	9	37	5	1.63	0.36	—0.11
4. Nicotine sulphate: 0.17% spray	2523	93	461	65	1.28	0.72	—0.13
5. Pestox III: 0.1%	628	59	75	5	1.01	—0.10	—0.60
6. Pestox III: 0.05%	2520	188	144	7	1.21	—0.27	—1.10
Coefficient of variation	0.5	0.4	0.4	0.5			
Difference for significance 5%					0.55	0.50	0.49
1%					0.73	0.66	0.66

TABLE II. TABLE OF MEANS, SPRAY TRIAL II.

Treatment	Mean no. live mites per sample		Differences between means of Transformed data 24 hr after treatment
	Before treatment	24 hr after treatment	
1. Check	854	881	0.02
2. Sulphur dust: 2.5g/plant	730	3	2.49
3. Midol 100 + oil: 0.1%	808	4	2.43
4. Arathane: 0.2%	746	0	2.85
5. Aramite: 0.15%	857	52	1.32
6. EPN-300: 0.1%	1090	10	2.09
7. Folidol: 0.1%	832	12	1.89
Coefficient of variation	0.4	0.6	
Differences for significance 5%			0.76
1%			1.04

TABLE III. TABLE OF MEANS, SPRAY TRIAL III.

Treatment	Mean no. live mites per sample		Differences between means of Transformed data 24 hr after treatment
	Before treatment	24 hr after treatment	
1. Check	619	475	0.14
2. Higam: 0.1%	687	95	0.86
3. Higam: 0.025%	620	86	0.93
4. Summer oil: 1%	385	50	1.12
5. Midol 100: 1%	510	1	2.45
Coefficient of variation	0.2	0.5	
Differences for significance 5%			0.66
1%			0.91

Table IV. Table of Means, Spray Trial IV.

Treatment	Mean no. live mites per sample		Differences between means of Transformed data 24 hr after treatment
	Before treatment	24 hr after treatment	
1. Check	420	577	—0.11
2. Aldrin: 0.1%	488	7	1.97
3. Dieldrin. 0.1%	701	136	0.68
4. Marlate: 0.1%	684	196	0.67
5. DD Temul: 0.1%	391	237	0.23
Coefficient of variation	0.2	0.4	
Differences for significance 5%			0.60
1%			0.83

Table V. Table of Means, Spray Trial V.

Treatment	Mean no. live mites per sample		Differences between means of Transformed data 24 hr after treatment
	Before treatment	24 hr after treatment	
1. Check	453	244	0.27
2. Midol T. 10: 1%	603	8	1.86
3. Neotran: 0.1%	351	119	0.61
4. P.P.C.: 0.1%	618	204	0.76
Coefficient of variation	0.2	0.4	
Differences for significance 5%			0.62
1%			0.87

TABLE VI. TABLE OF MEANS, SPRAY TRIAL VI.

Treatment	Mean no. live mites per sample		Differences between means of Transformed data 24 hr after treatment
	Before treatment	24 hr after treatment	
1. Check	240	563	—0.12
2. Nicotine sulphate: 0.17% + soft soap	273	0	2.33
3. Micronized sulphur dust: 2.5g/plant	254	0	2.30
4. Midol 100 + oil: 0.1%	250	0	2.37
5. Midol 100: 0.1%	248	0	2.38
6. Midol T. 10: 0.1%	248	2	2.02
7. Aldrin: 0.1%	257	0	2.41
8. Pestox III: 0.1%	260	3	1.91
Coefficient of variation	0.2	0.8	
Differences for significance 5%			0.43
1%			0.58

DISCUSSION AND CONCLUSION

Treatments within trials may be compared but not those between trials as variation due to differences in test method and environmental conditions between trials render such comparisons invalid. The following treatments were not significantly different from their check after 24 hours and may thus be regarded as ineffective:

DDTemul	0.1	per cent spray
Neotran	0.1	,, ,, ,,
P.P.C.	0.1	,, ,, ,,

The following treatments were near the borderline and may be regarded as of doubtful efficacy:

Higam	.1	and 0.25 per cent spray
Dieldrin	0.1	per cent spray
Marlate	0.1	,, ,, ,,

In all other treatments the number of survivors after treatment was significantly less than in the checks (at the 1 per cent level of probability); viz.

Arathane	0.2	per cent spray
Micronized sulphur dust	5 g and 2.5 g	per plant
EPN-300	0.1	per cent spray
Aldrin	0.1	,, ,, ,,
Midol T.10	1	,, ,, ,,
Midol 100 + oil	1	,, ,, ,,
Midol 100	1	,, ,, ,,
Folidol	1	,, ,, ,,

Nicotine sulphate	0.17	„	„	„
Aramite	0.15	„	„	„
Summer oil	1	„	„	„
Pestox III	0.1 per cent or 0.05 per cent spray			

The potentially useful acaricides for the tomato eriophyid mite fall into the last group. Some of these therapeutant agents were highly toxic to the tomato mite, e.g., Arathane, sulphur dusts, Midols. Others appeared to exert some residual effect, e.g., Pestox III. Further investigation is necessary however before recommendations can be made for the economic use of the newer acaricides tested here. Human toxicity must be considered as well as compatibility with existing spray materials. Field-scale trials will also be required.

Acknowledgment

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CONTROL OF ONION THRIPS

I. PRELIMINARY SELECTION OF INSECTICIDES

By R. A. Harrison and H. Jacks, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 3 September 1952)

Summary

During the 1951-52 season, the effectiveness of nine insecticides was tested against *Thrips tabaci* Lind. on onions. The insecticides were used singly or in combination of non-residual and residual types.

In most cases, applications at seven-day intervals gave a greater degree of control than those at fourteen-day intervals.

The most effective insecticides in this trial were Folidol, Hexone plus Nexol, Hexone plus Trimol DDT, Isopestox and Trimol DDT.

Introduction

As thrips severely damage onion crops in New Zealand, a trial was laid down to select insecticides effective in controlling this pest.

Life History and Habits of the Thrips

The chief thrips species occurring on onions in New Zealand is *Thrip tabaci* Lind. Another unidentified species of thrip was recorded from the trial area, but very few specimens were found.

Thrips tabaci is a minute, slender insect about 1/25 in. long. Males which are wingless are very scarce and were not observed during the trial. Females are winged, very active and are capable of reproducing parthenogenetically. Eggs are laid singly in the tissue near the base of the leaves. From these eggs hatch small yellowish nymphs, the first of four nymphal stages. The first two nymphal stages feed at the base of the leaves in the throat or chit of the plant where they are well protected. The last two nymphal stages (sometimes called the prepupae and pupae) are passed in the soil at the base of the plant. Adult thrips feed over the entire leaf. The insect feeds by puncturing the leaf surface with its mouth parts and imbibing the exuding sap. Damage to the leaf surface causes white blotches and streaks and lowers the vitality of the plant.

The number of thrips generations occurring in one season in New Zealand is not known. During the summer of 1951-52, adult thrips collected from the trial area were caged on onion plants in the glasshouse. Records taken at intervals showed that the minimum time required for adults of a successive generation to appear was 23 days. If this or a similar time is required in the field, as many as six generations could occur in any one season.

Materials and Methods

The following insecticides were used:

Nicotine sulphate (40 per cent nicotine), a water miscible preparation;

Nexol (16 per cent lindane), an emulsifiable preparation;

Trimol-DDT (25 per cent *p*-*p*^{1}-i.), an emulsifiable preparation;

Hexone (15 to 20 per cent tetraethylpyrophosphate), a water miscible preparation;

Midol T10, an emulsifiable preparation containing 2.7 per cent B.H.C. (0.7 per cent γ isomer) + 4 per cent azobenzene + 0.3 per cent parathion;

Summer oil, a petroleum oil emulsion of viscosity 80 (Redwood I at 100°F.) and unsulphonated residue of 95 per cent;

Folidol (25 per cent dichloryl-p-nitrophenyl thiophosphate), an emulsifiable preparation of parathion;

Pestox III (66 per cent bis-dimethylamino phosphonous anyhydride), a water miscible preparation;

Isopestox (90 per cent bis-monoisopropylamino fluorophosphine oxide); a water miscible preparation.

Non-residual substances, e.g., nicotine sulphate or Hexone, were applied separately or in combination with residual substances, e.g., DDT, lindane, summer oil or organic phosphorous compounds.

A field of seedling onions (variety, Pukekohe Longkeeper) was divided into four blocks. Each block was subdivided into 28 test plots, consisting of two adjacent rows of onions 10 yd. long. Test plots were separated by a buffer plot of the same size. There were 28 treatments and each occurred once in each block. Treatments were randomized throughout the four blocks. Two treatments of nicotine sulphate plus soft soap were added after the experiment was laid out. The four plots for each of these treatments were placed adjacent to one of the blocks.

With the exception of Pestox III, treatments were applied at either 7- or 14-day intervals. Pestox III, either alone or in combination with Hexone, was applied at either 14- or 28-day intervals. Applications were made with knapsack sprayers at the rate of 1 gal per treatment (240 linear feet of onions).

On 9 November 1951, when onion plants were first out of the seedling stage and 6-9 in. high, spraying was commenced and was continued until 15 February 1952. The onions were harvested on 26 February 1952. The stand of plants was not thinned throughout the experiment.

The thrips population was recorded on 19-20 December 1951 and again on 16-17 January 1952. Twenty-five adjacent plants from the centre of each treatment plot were examined on each occasion. Thrips on the three innermost leaves were counted.

When the onions were harvested, the total crop weight for each plot was recorded.

Results were submitted to statistical analyses.

Although the treatments with nicotine sulphate plus soft soap were not included in the blocks of randomized plots, the results of these are compared with the other treatments. This could be done, since the block difference did not even approach significance and thus no error would be introduced by comparing them with other treatments.

RESULTS

Results are given in the Table. Column A + B is used as a measure of the efficiency of a treatment. It is assumed that the effect of time is additive logarithmically, i.e. the effect of time is to alter the number of thrips by a given percentage rather than by a given number. This was substantiated by the fact that the interaction of treatments with the time was not significant.

Of the 7-day treatments, Nos. 1, 2, 4, 5, 6 and 9 are significantly more effective at the 5 per cent level than the 14-day applications of the same insecticides, viz. Nos. 10, 11, 20, 12, 17 and 19 respectively. The remaining 7-day treatments, viz. Nos. 3, 7, 13, 15, 21 and 26, however, are not significantly different at the 5 per cent level from the 14-day applications of the same insecticides, viz. Nos. 8, 14, 16, 24, 30 and 25 respectively. The Pestox III treatments at intervals of 14 days, viz. Nos. 18 and 23, do not differ significantly from the corresponding 28-day treatment, viz. Nos. 22 and 27.

RESULTS OF PRELIMINARY TESTS WITH INSECTICIDES AGAINST THRIPS TABACI ON ONIONS DURING 1951-52 SEASON
(n = number of thrips per treatment replicate)

Treatment		Conc. in 100 gal.	Period between applications (days)	Mean log (n + 1) for Dec. count A	Equiv. no. of thrips	Mean log (n + 1) for Jan. count B	Equiv. no. of thrips	A + B
No.	Name							
1	Folidol	1 lb	7	1.36	22	1.75	55	3.11
2	Hexone + Nexol	1 pt + 1.5 lb	7	1.23	16	1.98	95	3.21
3	Hexone + Trimol DDT	1 pt + 2.5 lb	7	1.26	17	2.06	114	3.32
4	Isopestox	1 lb	7	1.41	25	1.98	95	3.39
5	Trimol DDT	2.5 lb	7	1.42	25	1.99	97	3.41
6	Hexone + Isopestox	1 pt + 1 lb	7	1.59	38	2.18	150	3.77
7	Nexol	1.5 lb	7	1.44	27	2.36	228	3.80
8	Hexone + Trimol DDT	1 pt + 2.5 lb	14	1.42	25	2.43	268	3.85
9	Hexone	1 pt	7	1.36	22	2.56	362	3.92
10	Folidol	1 lb	14	1.41	25	2.52	330	3.93
11	Hexone + Nexol	1 pt + 1.5 lb	14	1.49	30	2.47	294	3.96
12	Trimol DDT	2.5 lb	14	1.45	27	2.59	388	4.04
13	Nicotine Sulphate + soft soap	1.3 pt + 5 lb	7	1.53	33	2.57	371	4.10
14	Nexol	1.5 lb	14	1.63	42	2.52	330	4.15
15	Midol T10	1 gal.	7	1.77	58	2.56	362	4.33
16	Nicotine Sulphate + soft soap	1.3 pt + 5 lb	14	1.66	45	2.67	467	4.33
17	Hexone + Isopestox	1 pt + 1 lb	14	1.65	44	2.68	478	4.33
18	Hexone + Pestox III	1 pt + 1 lb	14	1.52	32	2.88	758	4.40
19	Hexone	1 pt	14	1.79	61	2.70	500	4.50
20	Isopestox	1 lb	14	1.74	54	2.79	616	4.53
21	Nicotine Sulphate + summer oil	1.3 pt + 1.2 gal	7	1.90	78	2.69	489	4.59
22	Hexone + Pestox III	1 pt + 1 lb	28	1.91	80	2.71	512	4.62
23	Pestox III	1 lb	14	1.91	80	2.76	574	4.67
24	Midol T10	1 gal.	14	1.94	86	2.76	574	4.70
25	Summer Oil	1.2 gal.	14	2.03	106	2.80	630	4.83
26	Summer Oil	1.2 gal.	7	2.02	104	2.82	660	4.84
27	Pestox III	1 lb	28	2.05	111	2.85	707	4.90
28	Control-Water spray		14	2.03	106	2.86	723	4.89
29	Control-Water spray		7	2.13	134	2.79	616	4.92
30	Nicotine Sulphate + summer oil	1.3 pt + 1.2 gal	14	2.10	125	2.92	831	5.02
Difference required for significance at 5% level				.33		.44		.55
" " " " 1% "				.44		.58		.73

Discussion

From column A + B in the table it is seen that, with the exception of the summer oil treatments, all insecticides or combinations of insecticides show greater efficiency when applied at 7-day intervals than at 14-day intervals. In the case of the Pestox III treatments, the 14-day applications were better than the 28-day applications. In six cases the difference is significant at the 5 per cent level.

The insecticides which are most effective at intervals of 7 days tended to be the most effective at intervals of 14 days. For example, Folidol, Hexone plus Nexol, and Hexone plus Trimol DDT treatments are the most effective of the 7-day applications as well as the 14-day applications, although the order is not exactly the same. Similar comparisons can be made with the treatments of lesser efficiency.

From the table, it is also apparent that the greatest numerical difference between two consecutive treatments occurs between Treatments 5 and 6. Tukey (1949), who compared individual means in an analysis of variance, proposed a procedure for dividing treatments into distinguishable groups. According to his method the difference between Treatments 5 and 6 is more than significant at the 5 per cent level. Thus Folidol, Hexone plus Nexol, Hexone plus Trimol DDT, Isopestox and Trimol DDT fall into a group which may be distinguished as being the most effective in this trial.

The crop weight failed to distinguish between treatments. This was probably due to the fact that at no time was the crop thinned. Thus, in areas of poor germination bulbs were larger than in overcrowded areas and large differences occurred between the mean weight per plant per plot.

Acknowledgments

The authors' thanks are extended to Mr. D. McDougall, of Pukekohe, for providing help and facilities for this trial and to Mr. C. Dawe, Department of Agriculture, Pukekohe, for continued assistance throughout the experiment.

Statistical analyses of results were carried out by Mr. G. C. Ward, Applied Mathematics Laboratory, Department of Scientific and Industrial Research.

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CONTROL OF BACTERIAL CANKER OF TOMATOES (*Corynebacterium michiganense* (Erw. Smith) Jensen)

By D. W. Dye, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 22 July 1952)

Summary

In the 1951-52 season, dwarf tomato plants infected with bacterial canker (*Corynebacterium michiganense*) were sprayed with Bordeaux mixture, Cuprox, Perenox, Ascospray, and Phygon X.L.

Phygon X.L. markedly reduced fruit infection. All other materials were ineffective.

Yield was not affected by Phygon X.L., Cuprox, Perenox, or Ascospray but was considerably reduced by Bordeaux treatments.

Introduction

Bacterial canker of tomatoes (*Corynebacterium michiganense* (Erw. Smith) Jensen) causes extensive losses of plants and fruit of dwarf and to a lesser extent, staked tomatoes. The present recommendations for control are:

(1) Acid extraction of seed to eliminate seed-borne infection (Fry and Hastings, 1951);

(2) Soil disinfection of nursery beds and glasshouses;

(3) Planting out of only disease-free plants.

Little work has been done on the use of spray treatments to control bacterial canker of tomatoes. The present paper deals with the results of a trial using several therapeutant sprays in the 1951-52 season.

Experimental Methods

In September 1951, seedling tomato plants of the variety South Australian Dwarf were pricked out into boxes. During the period 1 to 24 October, they were infected with the canker organism *C. michiganense*. The seedlings were planted in the field on 1 November. The disease did not spread to new growth as rapidly as is usual under field conditions, and to obtain greater infection plants were sprayed with a suspension of the bacterial canker organism on 28 November and 3 December. Counts of infected plants, determined by presence of leaf lesions, were made prior to application of therapeutant sprays. Treatments, each of which included five replications, were randomized in blocks of 20 plants. Treatments were as follows:

(1) Bordeaux spray: 6 lb copper sulphate, 8 lb hydrated lime in 100 gal water.

(2) Bordeaux spray: 10 lb copper sulphate, 10 lb hydrated lime in 100 gal water.

(3) Cuprox (a proprietary copper oxychloride containing 50 per cent copper): 5 lb in 100 gal water, plus P.E.P.S. a proprietary compound containing 40 per cent polyethylene polysulphide, ½ lb in 100 gal water.

(4) Perenox: a proprietary cuprous oxide containing 50 per cent copper, 5 lb in 100 gal water.

(5) Ascospray: a proprietary mixture containing 2½ per cent phenyl mercury chloride, 1 lb in 100 gal water.

(6) Phygon X.L.: a proprietary mixture containing 50 per cent 2,3-dichloro-1,4-naphthoquinone, 1 lb in 100 gal water.

(7) Control: not sprayed.

Sprays were applied at 7-day intervals from 14 November 1951 until 27 February 1952. During this period, rain fell on 60 days, 11.43 in. being officially recorded in the trial area. Harvesting commenced on 25 January and continued at 7-day intervals until February 29, the total number of fruits and the number of infected fruits* being recorded for each block.

*Any fruit showing one or more canker lesions was recorded as "infected." No attempt was made to record severity of infection

RESULTS

A count of infected plants prior to treatment showed that 72 per cent were infected with bacterial canker and that they were evenly distributed over the area. The results of treatments are shown in Table I.

TABLE I. THE EFFECT OF VARIOUS THERAPEUTANTS ON CONTROL OF BACTERIAL CANKER ON TOMATO FRUIT

Treatment		Yield: Number of Fruit	Infected Fruit (%)	Means of Equivalent Angles
1. Bordeaux, 6-8-100		2864	45.6	42.4
2. Bordeaux, 10-10-100		2693	45.2	42.2
3. Cuprox, 5 lb plus P.E.P.S. ½ lb per 100 gal.		3885	26.1	30.7
4. Perenox, 5 lb per 100 gal.		3913	28.4	32.1
5. Ascospray, 1 lb per 100 gal.		3923	12.8	20.9
6. Phygon X.L., 1 lb per 100 gal.		4414	5.3	13.0
7. Controls		4034	15.6	23.1
Difference necessary for significance	5%	693		4.15
	1%	938		5.63

DISCUSSION

Phygon X.L. markedly reduced canker infection whereas the use of copper sprays led to a considerable increase in infection. Since 11.43 in. of rain fell on the trial area on 60 out of 106 days in the treatment period, it seems unlikely that this increase could be caused by increased humidity about the treated plants compared with the unsprayed controls. Bordeaux treatments, compared with all other treatments and with control, markedly reduced yields. If the incidence of canker infection is influenced by spray damage it is possible that weak Bordeaux treatments causing little plant damage could be effective against bacterial canker, although strong Bordeaux increased infection.

The above results are based on the work of only one season, but the control of bacterial canker of tomatoes by Phygon X.L. is sufficiently encouraging to justify large field scale tests.

ACKNOWLEDGMENT

Acknowledgment is made of assistance given with statistical analysis of results by Mr. G. C. Ward, Applied Mathematics Laboratory, Department of Scientific and Industrial Research.

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DIGESTIBILITY TRIALS ON SINGLE AND COMBINED FODDERS

1. SWEET LUPINS AND RAPE

By R. M. Allison and W. G. Thurston, Grasslands Division, Department of Scientific and Industrial Research, Lincoln.

(Received for publication, 12 May 1952)

Summary

Digestibility trials on sweet lupins and rape grown and fed separately and in combination are reported.

Statistical analysis and the combination of errors derived from yield and digestibility data has enabled comparisons of nutritive value per acre to be made between the crops.

Combinations of sweet lupins and rape have been shown to be much superior to any of the crops grown and fed separately.

Introduction

Supplementary fodder crops are used extensively in New Zealand and especially in Canterbury, both for fattening store lambs and also for feeding adult stock. Rape has always been popular for these purposes—the A. and P. Statistics for 1948-49 show that the area in rape was 163,829 acres, of which 85,756 acres, or 53 per cent were grown in Canterbury. However, the susceptibility of rape to aphis attack, especially under the hot, dry conditions frequently experienced in Canterbury, is a major defect. Much interest has, therefore, been created by the introduction of sweet varieties of lupins which appear to offer marked advantages as summer forage crops.

Little quantitative information is available on the relative yields and feeding values of rape and any of the available sweet lupin varieties in New Zealand, except for a lamb-fattening trial conducted at Canterbury Agricultural College[1], and yield and digestibility trials by Sears and Black[2]. Advantage was therefore taken of a field trial conducted by the Agronomy Division*, Department Scientific and Industrial Research, Lincoln, to gain information on the digestibility coefficients and yields of rape, sweet blue, sweet yellow and sweet white lupins grown and fed separately and in legume/non-legume association. Sweet white lupin was, however, not included in the latter mixture since it is a flower colour mutant of sweet blue lupin and was considered unlikely to differ markedly from sweet blue in other respects.

The rape used was Broad Leaf Essex II (B.L.E. II). This variety was selected by the Agronomy Division from the progeny of a backcross (Broad Leaf Essex × Giant Choumoellier) × Broad Leaf Essex. The selection proved superior in agronomic characteristics to either parent. The sweet blue lupin used was introduced from Europe by the Agronomy Division in 1938; the sweet white lupin was a mutant selected from this line by the same institution. The sweet yellow lupin was introduced by a Canterbury farmer in 1937; seed from this line was obtained from a seed merchant and purified by the Agronomy Division.

This paper records yield and digestibility figures for the rape and lupin varieties described above, grown and fed singly and in combination, and fed at two different stages of growth.

* Now Crop Research Division.

Experimental Technique

The crops were sown on an area from which a crop of Dreadnought wheat had been taken the previous year. Green manure was sown after the wheat had been harvested and this was ploughed under in early spring. Weather conditions which delayed cultivation, prevented the crops being sown till late in November, 1946. The field trial consisted of three replications of which the third provided the material for the digestibility trial.

Superphosphate fertilizer was first applied at the rate of 1 cwt. per acre, after which the crops were sown with a Planet Junior seed sower in rows 1 ft. apart. The plots were 22 ft. by 152 ft. and contained 21 rows. The seeding rate for the lupins was 3 bushels per acre throughout; that for the rape was 2½ lb per acre. Combinations were sown at these rates also i.e. 2½ lb of rape per acre plus 3 bushels of lupin per acre, the rape being drilled first followed by lupins in the same rows. Early establishment and growth were good, but weed invasion, mainly fathen (*Chenopodium album*) and spurrey (*Spergula arvensis*) soon became serious. Growth conditions were good; earth temperatures were average for the period[3], and the rainfall was above average[4 5].

The sheep used in the trials were 6-tooth Romney wethers which had previously been used in outdoor grazing trials. They were housed in separate stalls indoors and the faeces were collected in bags[6]. For each trial a three-day prefeeding period was followed by a five-day measurement period. As the crops were severely infested with weeds, these were separated and only the pure feed was offered to the sheep. A sample of each feed was taken daily from the weed free material and a composite for chemical analysis was made from these samples by aliquot on the basis of dry matter consumed. The residue from each sheep for each day's feeding was weighed and sampled and a composite for each sheep prepared on the basis of the daily residue weight. Separate chemical analyses for each sheep were necessitated by the different selective feeding of the animals. Faeces samples were collected daily and composite samples for each sheep were prepared in the same manner.

The sheep were fed to appetite and, with the exception of Sheep No. 6 in the first trial, each pair behaved in a reasonably similar and normal manner. It should be noted that sheep No. 6 was replaced by No. 13 in the second trial. The botanical analyses shown in Table I are the averages of daily random 500-g samples taken from each feed as cut from the field. After separation into component species, the samples were dried in an electric oven for 24 hours at 80°c. The percentage compositions were calculated on the basis of dry matter. The crops were sampled for yield in the following manner:—For each day of the trial, three samples each 2 ft. × 5 rows were cut and weighed green. Since each trial consisted of a five-day feeding period, there were thus five such samplings taken from each of the three replications of the field trial. Each daily set of three samples was bulked, thoroughly mixed, and a 500-g aliquot was separated into pure feed and weeds and dried. For each trial the dry matter yields of weed free crop were then calculated on a per acre basis from the replication means of the pure feed

analyses. From the daily replicates 500-g samples of pure feed were dissected and dried. The percentage dry matter content of the pure feed as calculated for the trial period, was the mean of the five daily replications.

RESULTS

Trial I—7 to 11 February, 1947.

Table I (a) shows the amounts of Dry Matter (D.M.) eaten by each sheep in the period, together with a description of the crop.

The maturity of the rape was judged to be a week past the acceptable optimum. In the lupins, flower buds were more numerous on the sweet white and sweet blue varieties than on the sweet yellow.

Trial II—27 February to 3 March, 1947.

Table I (b) provides similar data for the second trial.

The main points of interest were the increase in growth of sweet yellow lupins relative to the other two varieties, and the absence of disease and pest damage on that crop. "Sore shin," the disease affecting the sweet white lupin and the sweet blue lupin, is a virus disease caused by the virus of pea mosaic[7].

The maturity of the rape appeared to be well past the accepted optimum stage for feeding. The bottom leaves were yellow and some had dropped off.

TABLE I. DRY MATTER CONSUMED BY SHEEP FEEDING ON LUPINS AND LUPINS PLUS RAPE AT DIFFERENT STAGES OF MATURITY

Crop	No. Sheep	D.M. consumed (lb)	Height of crop (in.)	Botanical Analysis (% D.M.)			Condition of Crop			
				Lupins	Rape	O.S.**	Crop	Disease	Pest	Maturity
(a) Trial 1. 7/2/47 - 11/2/47.										
Sweet White Lupin	1	17.3	16-17	76	-	24	Lupins	-	-	Flower buds
	2	17.9								No colour
Sweet Yellow Lupin	3	12.8	16-17	59	-	41	Lupins	-	-	Flower buds
	4	12.0								No colour
Sweet Blue Lupin	11	17.3	16-17	71	-	29	Lupins	-	-	Flower buds
	12	16.4								No colour
Sweet Blue Lupin	5	10.0	16-17	59	38	3	Lupins	-	-	Buds and colour
+ Rape	*6	2.8	10				Rape	-	-	Over-mature
Sweet Yellow Lupin	7	12.4	14-16	36	61	3	Lupins	-	-	Buds no colour
+ Rape	8	12.9	10				Rape	-	-	Over-mature
BLE II Rape	9	13.0	10	-	92	8	Rape	-	-	Over-mature
	10	13.4								
(b) Trial 2. 27/2/47 - 3/3/47										
Sweet White Lupin	1	14.4	24-25	54	-	46	Lupins	+	++	Green pod
	2	13.0								
Sweet Yellow Lupin	3	14.2	27	72	-	38	Lupins	-	-	Green pod
	4	12.8								
Sweet Blue Lupin	11	16.2	23	69	-	31	Lupins	+	++	Green pod
	12	15.3								
Sweet Blue Lupin	5	11.7	23	55	43	2	Lupins	+	++	Green pod
+ Rape	13	12.2	12				Rape	-	+	+ Over-mature
Sweet Yellow Lupin	7	13.6	23	36	61	3	Lupins	-	-	Green pod
+ Rape	8	15.7	12				Rape	-	+	+ Over-mature
BLE II Rape	9	10.7	11	-	90	10	Rape	-	+	+ Over-mature
	10	12.1								

* replaced in Trial 2.
** Other species mainly fat hen and spurry

- indicates freedom from disease or pest
+ indicates slight infection by disease or pest
++ indicates severe infection by disease or pest

Composition and Nutritive Value of the Feeds

Chemical analyses of feeds, faeces and residues were performed according to the standard procedures given in the A.O.A.C. Methods of Analysis[8], 1945 for stock feeds. Criticism of the Henneberg method of evaluating feeds[9] is admitted, but it was felt that no alternative was available which would be easy to apply and conform to the requirements of comparability with past work.

The chemical composition and nutritive value of the feeds and the yields of nutrients per acre, are shown in Tables II and III.

[illegible]

Feed	Rate											
Sweet White Lupin							.4 .6					
Sweet Yellow Lupin							.3 .9					
Sweet Blue Lupin							.4 .7					
Sweet Yellow Lupin + Rape RLE II							.0 .5					
Sweet Blue Lupin + Rape RLE II							.5 .0					
Rape RLE II							.8 .9					

* The differences between the means for trials was not statistically significant.

TABLE III. YIELDS PER ACRE OF DRY MATTER

Feed	Trial	D.M. per acre of weed free feed (lb)	Dry starch equivalent per acre (lb)	Dry digestible protein per acre (lb)	Nutritive ratios
Sweet White Lupin	I	2635	1489	421	3.55
	II	2433	1161	365	3.18
Sweet Yellow Lupin	I	1571	985	219	4.50
	II	3334	1880	449	4.20
Sweet Blue Lupin	I	1978	1165	292	4.00
	II	2485	1317	360	3.67
Sweet Blue Lupin + Rape	I	3820	2635*	441*	5.95*
	II	4492	2834	463	6.10
Sweet Yellow Lupin + Rape	I	3614	2382	287	8.30
	II	3894	2457	260	9.45
Rape	I	2832	2116	197	10.4
	II	2815	1959	175	11.1
		± 149 Sig.diff 1% 602 " " 5% 383	± 103 Sig.diff 1% 415 " " 5% 265	± 22.4 Sig.diff 1% 90 " " 5% 58	

* One sheep only used.

DISCUSSION

Field Observations

Of the lupin varieties, it was most apparent that sweet yellow lupins took longer to reach maturity and maximum production (Table I) than either sweet white or sweet blue, which were very similar. This later maturity was borne out by D.M. yields and the figures for starch equivalent and digestible protein per acre (Table III).

The observations on disease damage and insect infestation also showed differences. Sweet white and sweet blue lupins were more severely affected by "sore shin" and thrips when sown with rape, but were still fairly severely affected when sown alone. The sweet yellow variety, however, showed no signs of damage from either disease or pest. This indicates greater resistance, which would be a definite factor in favour of sweet yellow lupins in areas where disease or insect damage are likely to occur.

Where rape and lupins were sown in combination, higher D.M. yields (Table III) were obtained than where either crop was sown alone, in spite of the fact that it was obvious that the lupins suffered from competition by the rape. The sweet yellow variety was more affected in this way than the sweet blue. This could probably be accounted for by the later maturity of sweet yellow lupins and the consequent greater susceptibility of this variety to competition by rape.

Another point of interest arising from the use of the mixture of rape and lupins was that weed invasion was much less severe than where the crops were sown alone. In Trial 1 (see Table I) there was 41 per cent of weeds in the sweet yellow lupin crop, and 29 per cent in the sweet blue lupins. Where these varieties were sown with rape, the figure for other species was reduced to 3 per cent in both cases. Similar results are apparent for Trial II. The differences were not so great in the rape crop. Probably the earlier cover afforded by the rape plants tended to reduce the growth of weeds. Where spring weeds are a menace to crop establishment, as in Canterbury, this factor is of prime importance.

It was obvious in this trial that rape sown on the same day as lupins reached maturity approximately two weeks earlier. However, where the rape was sown in combination with lupins, it did maintain its freshness better than when sown alone. Unfortunately, from a practical point of view, it would be difficult to sow the combination other than at the same time.

Digestibility Results

Comparison of the results in Table II with those of other experiments in Canterbury[9, 10] indicates general agreement. Unpublished data on sweet blue, sweet yellow, and bitter blue lupins grown at Palmerston North[2] indicate, on the other hand, considerable differences. Any conclusion arising from the present investigation must therefore be accepted as applying only to Canterbury conditions.

Large differences in performance are apparent between the sweet lupins and rape of the present investigation (Table II). On a starch equivalent per cent, B.L.E. II. rape is by far the most concentrated feed. This must be considered in relation to the digestible crude protein when attempting to compare and evaluate the functions of the different single feeds. Thus, the widely differing nutritive ratios suggest different functions for lupins and rape. Final evaluation depends upon the extension of the data contained in Table II to a per acre basis. Statistical analysis of the yield and digestibility figures in Table II and III, and combination of the errors derived from these separate data has enabled this to be done, and valid comparisons between the feeds and trials may be made (Table III). The authors suggest that far greater value may be attached to digestibility trials if yield and digestibility data are combined in this way.

In Table III, the effect of yield on the relative nutritive capacities of the different fodders is immediately apparent. This is especially noteworthy in the cases of the combination fodders sweet blue lupin plus B.L.E. II rape, and sweet yellow lupin plus B.L.E. II rape. In these instances, increases in dry matter yield per acre of 25-50 per cent over single crops, and the resulting increases in starch equivalent yields, should be noted. Thus, in nutritive capacity, the combinations are much superior, although there is little to choose between the two combinations. Digestible protein per acre shows wide variability among all feeds, and it is here that a choice can perhaps be made between the two lupin

combinations as regards function. Another noteworthy feature to be seen in the table is the large increase in the yield of sweet yellow lupins between the first and second trials.

As has already been pointed out, sweet white lupins are a white flowered selection from sweet blue lupins. It is interesting to note from Tables II and III that the varieties differed considerably in chemical and nutritive characteristics.

These trials have shown conclusively the advantage to be gained by growing and feeding combination crops. Severe weed infestation was practically eliminated by the combinations. Whether the increased production noted was due to this cause alone, or whether the known advantageous association between legume and non-legume was a contributing factor, is not determinable from the present investigation. Most of the rapid effect of the association has been shown to be due to overground transfer, but this can be eliminated from present consideration since there was no grazing of the crops *in situ*. However, the possibility of underground transfer cannot be neglected and it is hoped to test this point.

Acknowledgements

The authors wish to acknowledge their indebtedness to Mr. G. M. Wright, Applied Mathematics Laboratory, Department Scientific Industrial Reseach, Lincoln, for statistically analysing the results; and to Mr. R. A. Belcher for assistance in running the digestibility trials.

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THE MEASUREMENT OF FEED INTAKE BY GRAZING CATTLE AND SHEEP

PART. II. THE DETERMINATION OF CHROMIC OXIDE, AND MONASTRAL BLUE IN COW FAECES

By M. R. Coup and R. J. Lancaster, Ruakura Animal Research Station, Hamilton, New Zealand

(Received for publication, 28 July, 1952)

Summary

1. Chemical methods are described for the estimation of chromic oxide and monastral blue in cow faeces.

2. Results of recovery tests are presented. These show that the errors of estimate were ± 1.5 per cent for the chromium estimation and ± 2 per cent for the monastral blue estimation.

Introduction

In the course of the development of a method for measuring the pasture intake of grazing cows, using chromium sesquioxide as a faeces reference substance, it has been necessary to undertake a considerable number of determinations of chromium in faeces. After trial of several other methods of estimation of chromium, including those described by Barnicoat[1], Maunsell[2] and De Lippa[3], a method based on that developed by Kolthoff and Sandell[4] for determination of chromium in steels has been adopted, because it could be applied as a routine to a large number of samples with the requisite speed and accuracy.

In certain of the intake measurement trials, monastral blue (copper phthalocyanin) was fed, in addition to chromium sesquioxide, as a second reference substance, and copper was determined in the faeces. It was found that the copper could not be satisfactorily determined in the presence of chromic oxide, and a separation of copper from chromium had to be effected before the assay was proceeded with. In this paper are presented details of the methods for determination of chromium in faeces, and of copper in faeces containing chromium sesquioxide, which have been used in the investigations described in later papers.

Determination of Chromium Sesquioxide in Faeces Samples

Procedure

Ashing of Sample: A sample consisting of about 0.5-0.8g of the dried ground faeces is ashed for 1½ h in a muffle furnace at about 580°c and then quantitatively transferred to a 150-ml Erlenmeyer flask using distilled water.

Oxidation: 12 ml of acid mixture and 1 ml of manganous sulphate solution are added and the volume made up to 40-50 ml. A 3-ml portion of potassium bromate solution is added, the flask is swirled and placed on a hot plate in a fume cupboard. At about 15 minute intervals further 3 ml portions of oxidising agent are added, to make 15 ml in all. Glass beads or porous pot are necessary to prevent superheating during the

oxidation. It is important to keep the volume at approximately 40-50 ml throughout the oxidation stage—for this purpose boiling water is added through a glass rosehead and thus the sides of the flask are washed down in the same process. Twenty minutes after the last addition of bromate 5 ml of $(NH_4)_2SO_4$ solution is added by tilt measure. (Bromine fumes are evolved copiously at this stage). Ten minutes later a further 5-ml portion of $(NH_4)_2SO_4$ is added, and when evolution of bromine ceases (tested with KI-starch papers) the volume is made up to over 60 ml and 1 ml of 2N HCl added. Boiling is continued until no further reaction is given with starch-iodide paper. Five minutes is usually sufficient, but it is advisable to test each flask individually with test paper as it is removed from the hot plate.

Reduction and back-titration: After cooling to room temperature, the mixture is diluted to about 80 ml and 5 ml of 2N HCl and 3-4 drops of N/400 KI are added. A measured volume of arsenious acid, 3 ml or 5 ml as required to give adequate excess, is run in and back titrated with permanganate. The end point causes little difficulty and is readily confirmed by the addition of ½-1 drop $KMnO_4$ excess.

Standardization: Both the permanganate and arsenious acid are standardized against exactly N/10 dichromate as follows: To 12 ml of acid mixture, diluted to 80 ml in a 150 ml flask are added 5 ml of 2N HCl and 3-4 drops N/400 KI. For "Arsenic standards" 3 ml or 5 ml amounts of arsenious acid (whichever is being used in the set of determinations) are run in; for "Dichromate standards," 2 ml of N/10 dichromate is added followed by either the 3 or 5 ml of arsenious acid. Both sets of standards are now titrated with $KMnO_4$.

Reagents: The following reagents were used:

Standard Potassium Dichromate: Exactly N/10 solution prepared from Analar potassium dichromate dried at 150° C.

Acid Mixture: One volume of conc. sulphuric acid, one volume phosphoric acid (S.G. 1.75) added to two volumes distilled water.

Manganous Sulphate Solution: 1 ml = 0.75 mg Mn.

Potassium Bromate: A 4½ per cent solution.

Ammonium Sulphate: A 20 per cent solution.

Arsenious Acid Solution: An approx. N/10 solution is prepared by dissolving 4.9 g of pure As_2O_3 in 80 ml of N. NaOH, diluting, acidifying with HCl and making to 1 litre.

Potassium Permanganate: Approx. N/50. It is convenient to prepare a 2N. stock solution, from which the dilute solution is prepared daily.

Discussion of Procedure

Throughout this work it was necessary to use potassium dichromate as ultimate standard as no pure chromium sesquioxide was available for this purpose. The chromic oxides used in all our field experiments have been commercial grades supplied as paint pigments under the name of chrome green. As different batches

of this material vary in purity, each batch has been assayed for its Cr_2O_3 content by the method described, the mean of a number of determinations being taken as the "Batch purity value". All our work would indicate that oxidation of Cr_2O_3 to CrO_3 is complete under the conditions used and that the impurities in the material as fed are not chromium compounds. Even if this is not so, the fact remains that the material of any batch gives a constant and reproducible Cr_2O_3 value by this method. The following values for replicate analysis of two different batches of chromium oxide serve to illustrate the "purity" determination:

Batch	Purity Values					Mean Purity Value
A	95.4	95.3	95.3	96.3		95.6
B	92.8	93.0	92.0	92.3	92.2	
		92.1	92.8	92.3	92.3	92.4

Such purity values have been used to calculate the amount of chromic oxide added in the recovery trials described later.

To establish the accuracy of this method as adapted for the determination of chromic oxide in faeces, recovery trials have been undertaken and various factors likely to affect the determination have been investigated.

Effect of range of Cr_2O_3 *Concentration on the Accuracy of Determination*

This was tested in two ways—at the volumetric stage only with pure solutions of N/10 potassium dichromate, and over both oxidation and volumetric stages with both dichromate and sesquioxide. The amounts of chromium (as Cr_2O_3) present in these tests ranged from 5 mg to 25 mg, and satisfactory recoveries were obtained at all levels in all cases.

The Influence of the Amount of HCl *Added at the end of Oxidation*

These tests were carried out using N/10 dichromate solutions. It was found that 1 ml of 2N HCl was generally adequate to remove last traces of bromate, although the addition of up to 10 ml did not affect the result.

Effect of the use of $MnSO_4$ *as Catalyst in the Oxidation*

It was found that small amounts of manganese considerably speeded up the oxidation and allowed it to proceed at higher dilutions. Since the amount of manganese present in faeces is likely to be variable, addition of $MnSO_4$ was introduced as a regular procedure prior to the start of oxidation. Tests with and without manganese, both on pure solutions and in presence of faeces, showed that the $MnSO_4$ did not significantly affect the final Cr_2O_3 recoveries.

Recovery Tests with Dichromate added to Faeces

In some of these tests, known amounts of potassium dichromate were added to dried ground faeces, while in others the dichromate was added after the faeces had been ashed. At the same time, determination on pure solutions taken through both the oxidation and volumetric stages, were carried out. The results, expressed as percentage recoveries of the Cr_2O_3 added, are summarized as follows:—

(a) Pure solutions: 99.5 per cent, 100.2 per cent

(b) Dichromate added to ash: 99.8 per cent, 100.8 per cent

(c) Dichromate, ashed with ground faeces: 99.4 per cent.

Recovery Tests with Chromic Oxide added to Faeces

In these trials, four grades of commercial chromic oxide and one sample prepared in the laboratory from ammonium dichromate were added to faeces, either by weighing the oxide (on a micro balance) directly into weighed portions of fresh or dried, milled faeces, or by mixing the oxide with a large bulk of fresh faeces and taking subsamples of this mix for analysis.

After ashing, both types of sample were analysed for Cr_2O_3 content. The results are summarized in Table I. In the column headed "Cr_2O_3 content of oxides" are given the Cr_2O_3 assays of the chromium oxides added and the results of analyses of the oxide—faeces mixtures. Both these values are expressed as mean percentage values with standard deviations. These mean percentages were used to calculate the recoveries given in the last column.

Table I. Chromic Oxide Recoveries

Method of Addition	Chromium Oxide Sample	Cr_2O_3 Content of Oxides		% Recovery
		Without Faeces (%)	With Faeces (%)	
1. To portions of dried faeces	A	92.4±0.3(9)*	92.6±0.5(6)	100.3
	Lab. Prep.	98.5±0.3(6)	98.4±0.7(5)	99.9
2. To portions of fresh faeces	B	95.6±0.5(4)	95.3±0.7(8)	99.7
	C	86.6±0.6(6)	87.8±0.4(4)	101.5
	D	93.2±0.8(6)	93.8±1.2(4)	100.7
3. To one large sample of fresh faeces, mixed and subsampled — Mix 1	C	86.6±0.6(6)	86.3 (9)	99.7
Mix 2	D	93.2±0.8(6)	92.2 (9)	98.9
Mix 3†			92.4 (4)	99.1
Mix 4			92.8 (4)	99.6

*Figures in brackets indicate numbers of individual determinations

†Mix 3 and 4 contained monastral blue in addition to chromic oxide.

The following comments may be made from the data in Table I:

(a) A high degree of precision of the method has been demonstrated.

(b) The amount of Cr_2O_3 found in a given sample was unaffected by the presence of faeces or monastral blue, i.e. the recovery of chromium sesquioxide was constant under the various conditions imposed.

(c) Mixing the oxide with a mass of faeces and subsampling includes, in addition to any sources of error inherent in the chemical procedure, other factors such as the efficiency of mixing of chromic oxide with faeces, and errors in deter-

mination of dry weight. Since these are all sources of error in determination of Cr_2O_3 in faeces from animals dosed with chromic oxide, the recovery results obtained by this method of mixing are of particular interest, and indicate the suitability of the method for application in a routine intake measurement procedure.

In conclusion it may be stated that:

(1) None of the modifications used were found to have introduced any source of error;

(2) The divergences from the theoretical values throughout the recovery trials and other tests have in almost all cases been well within ± 1.5 per cent.

This method of chromic oxide determination has now been successfully used over a number of years for both reference substance recovery trials and for routine intake measurements of a large dairy herd. The results of these investigations will appear in subsequent papers.

ESTIMATION OF MONASTRAL BLUE IN COW FAECES CONTAINING CHROMIUM SESQUIOXIDE

Since monastral blue (copper phthalocyanin) contains 11.02 per cent of copper, its concentration in faeces can be readily followed by copper estimation. It was originally proposed to estimate the copper by the method described by Clare *et al*[5], but it was found that when faeces samples containing chromium sesquioxide were analysed by this method, recoveries of copper were some ten per cent too low. The low recoveries were traced to chromium interference, explainable on the assumption that both copper and chromium, brought into solution by the faeces digestion procedure, would react to form insoluble copper chromates. Separation of copper from chromium had first to be effected, and the method adopted to carry this out is described below.

Method 1

Apparatus and Solutions

Graduated Florence Flasks: About one hundred 50-ml pyrex florence flasks were calibrated with a mark on the neck to measure a known quantity of liquid. These flasks are used for carrying out the faeces extraction, and for making the extract to volume.

Extraction Mixture: 800 ml glacial acetic acid and 200 ml concentrated nitric acid.

Digestion Mixture: 200 ml concentrated sulphuric acid, 40 g anhydrous potassium sulphate, made to 1 litre with distilled water.

Buffer Solution: 200 ml 50 per cent ammonium citrate solution, 500 ml ammonia solution (S.G. 0.880) made to 1 litre.

Colour Reagent: 0.1 g sodium diethyldithiocarbamate is dissolved in 50 ml buffer solution, and made to 100 ml with ammonia solution (S.G., 0.910).

Filtering Tubes: To remove moisture droplets, the amyl alcohol extracts are filtered through cotton wool pads inserted into 15 × 100 mm glass tubes drawn to a constriction at one end. To free it from copper, the cotton wool is extracted with cold 5 per cent nitric acid (24 hr) filtered and washed with distilled water until acid free. It is then

washed with copper-free ethyl alcohol, and dried at 95°c.

After filtration, copper-free ethyl alcohol (20-30 ml) is passed through each tube, which is dried, and is ready for use again. One cotton wool pad could be used for at least a dozen filtrations.

Procedure

About 0.8 g of ground faeces samples is introduced into a calibrated florence flask, 10 ml of the "extraction mixture" added, and the flask is boiled quietly on a hotplate for 30 min. The contents, on cooling, are made to volume, mixed and stood overnight.

Five ml of the supernatant liquid is transferred to 8 in. x 1 in. pyrex test tubes, calibrated at 25 ml, containing 5 ml of "digestion mixture," a trace a selenium and two glass beads. The contents of the tubes are boiled for some 30 min. after "clearing," the total boiling time being about 1 hr. On cooling, the tubes are rinsed down with distilled water, 10 ml of "buffer solution" added and on cooling again, 2 ml of colour reagent is added. The contents are made to 25 ml with distilled water. The solution is extracted with 10 ml isoamyl alcohol, of which 5-6 ml is filtered through the filtering tubes into test tubes.

The colour of the amyl alcohol solution is measured at 448 μ in a spectrophotometer.

Recovery Tests

The above procedure was submitted to two series of tests in order to evaluate the accuracy of the estimations of monastral blue.

Series 1: Small amounts of monastral blue (range 1.11 to 4.93 mg) were weighed into graduated florence flasks containing 0.8g of dried ground faeces. In some tests, chromium sesquioxide (15 mg) was added, while this was omitted from others. The copper contained in the flasks was estimated by the procedure detailed above, and after allowing for the natural copper extractable from the faeces, the monastral blue present was calculated. The recovery of the pigment from ten tests was 99.66 ± 1.77 per cent.

These recovery tests were carried out on dried ground faeces to which the pigments had been added. Such conditions did not entirely represent the conditions obtaining when the pigments were first mixed with fresh faeces; hence, a further recovery experiment was carried out as follows:

Fresh cow dung was divided into six 1-kg samples. Monastral blue (0.5-1 g) was thoroughly mixed with each dung sample, whilst chromium sesquioxide was mixed with three of the samples. A second similar experiment, involving four subsamples of faeces, two containing monastral blue, and two containing both monastral blue and Cr_2O_3, was performed. The added chromium had no effect on the results, and the recovery from 10 experiments was 99.55 ± 1.88 per cent. The range of concentrations of monastral blue in faeces dry matter was 3.90 to 10.05 mg/g of dry matter.

Method 2

In a further series of experiments to be reported, monastral blue was used alone, as an inert reference substance, in the measurement of grass consumption by sheep. As copper and

nitrogen concentrations in faeces were the only data required in the estimation of grass intake by the sheep, a procedure was adopted which enabled both copper and nitrogen to be estimated in the same Kjeldahl digest of faeces.

Apparatus and Reagents

Calibrated Kjeldahl Flasks: Kjeldahl flasks were calibrated in the neck to contain 70 ml.

Buffered Carbamate: The sodium diethyldithiocarbamate is added to the "buffer solution" (Method 1) at the rate of 0.01 per cent.

Procedure

About 0.5g dried ground faeces is digested in calibrated Kjeldahl flasks in accordance with the instructions given by White *et al*[7]. The digests are made to the mark, and 5 ml portions are withdrawn for nitrogen and copper estimations.

For copper, the 5-ml sample is introduced into 6 in. x 1 in. test tubes containing 10 ml of "buffered carbamate". The copper complex is extracted with 10 ml of isoamyl alcohol, and the estimation is completed as described in Method 1.

Method 2 was subjected to recovery tests similar to those already described, yielding a standard error of estimate of less than ± 2 per cent.

Standardization. Standard copper extracts are prepared from monastral blue, by using the procedure appropriate to the method (1 or 2) in use.

Correction for Natural Copper. From the recovery data presented in this paper, it appears that the precision of the methods of estimation used is of the order ± 2 per cent, which is probably well within the faeces sampling error. It is possible that an additional source of error will be introduced through unexpected fluctuations in the concentration of natural copper in the faeces, for which allowance must be made in calculating the monastral-blue content in the faeces. If the monastral blue is dosed at such a level that its concentration in faeces is at least ten times that of natural copper, the proportionate error due to an undetermined fluctuation in natural copper of say 10 per cent would be only 1 per cent. In experiments so far conducted (three experiments involving 15 animals) the natural copper concentration (as monastral blue) ranged from 0.23 to 0.52 mg/g faeces dry matter. Most of the differences were found between faeces samples derived from different feeds, similar copper levels being recorded for faeces derived from the same feed. If, therefore, the concentration of monastral blue in faeces be maintained at or above 5 mg/g, errors due to fluctuations in natural copper are unlikely to be serious.

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PHOTOSENSITIVITY DISEASES IN NEW ZEALAND

VI. SOME LIVER FUNCTION TESTS ON CONGENITALLY PHOTOSENSITIVE SOUTHDOWN, NORMAL SOUTHDOWN, AND NORMAL ROMNEY SHEEP

By N. D. Jamieson and J. B. Swan, Animal Research Station, Department of Agriculture, Wallaceville

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Summary

The following liver function tests were carried out on normal Southdown, normal Romney and congenitally photosensitive Southdown sheep: serial rose bengal excretion, serial bromsulphthalein excretion, serum alkaline phosphatase level, plasma prothrombin clotting time, hippuric acid synthesis and water excretion.

Both the bromsulphthalein and the rose bengal tests gave similar results, the photosensitive Southdowns showing a marked inability to remove these dyes from the blood stream.

No significant differences were found in the serum alkaline phosphatase levels, and prothrombin clotting times for the normal and photosensitive Southdowns.

The photosensitive Southdowns exhibited a marked water retention after oral administration of water in conjunction with the hippuric acid synthesis test, and this is probably the cause of the slightly decreased recovery of hippuric acid found in these animals.

The normal Romney sheep gave results similar to those of the normal Southdowns for all of the tests.

Introduction

Evidence obtained by Hancock[1] from planned breeding experiments has shown that congenital photosensitivity in Southdowns depends on a single recessive factor with full penetrance.

Biochemical findings in the disease are: an abnormal porphyrin pigment metabolism, with evidence of a liver dysfunction as gauged by the Van den Bergh test for plasma bilirubin, and a failure of the liver to remove injected rose bengal from the blood stream[2]. Clare[3] found that phylloerythrin is the photosensitizing agent in the blood. There are, however, no gross histological abnormalities present in the liver in this condition[2], and celloidin casts prepared by the method of Swan[4] were normal.

The present work was undertaken in order to gain further information on the liver dysfunction present, by comparing the response of the photosensitive Southdown to various liver function tests with that of normal Southdown and Romney sheep. The following tests were chosen in order to cover a range of liver functions: serial rose bengal and serial bromsulphthalein (dye excretion), serum alkaline phosphatase level (secretion and excretion), prothrombin index (vitamin K absorption and utilization), and hippuric acid synthesis (glycine synthesis and conjugation).

Experimental Methods

The three photosensitive sheep were obtained from Ruakura Animal Research Station and were tested with three normal Southdowns from

the Wallaceville flock. The tests were also made on normal Romneys in order to assess any variation in normal values due to breed. The Ruakura sheep were from a known line of congenitally photosensitive animals and the presence of the abnormality was confirmed by: a positive Van den Bergh test; the failure to excrete more than 50 per cent of injected rose bengal 8 min. after injection; and the presence of a photosensitivity after eating pasture chlorophyll.

The photosensitive animals stayed of their own accord in covered pens during daylight and went out to graze in an adjacent paddock during the night, returning to shelter at daybreak. Except for the test periods, the normal sheep were in the same paddock all the time.

Serial Rose Bengal Excretion Test

Other workers have shown[2] that the photosensitive Southdown exhibits an abnormally low rate of excretion of injected rose bengal.

For the present work, a serial form of the test was used; samples were taken at 2, 5, 8, 12 and 20 min. after an intravenous injection into the jugular vein of 10 ml of a 1 per cent solution of rose bengal in normal saline; in order to obtain more detailed information on the rate of removal of the dyestuff from the blood stream, than that provided by the earlier method of measuring the excretion at 8 min. The level of dye in the samples was measured colorimetrically with the 2-min. sample taken as 100 per cent retention.

Serial Bromsulphthalein Excretion Test

A dose of 5 mg/kg body weight of phenol-tetra-bromphthalein sodium sulphonate was injected into the jugular vein, as a 1.25 per cent aqueous solution, over a period of 60 seconds. Zero time was recorded half-way through the injections and bleedings were made from the vein on the opposite side at 2, 4, 7, 10, 15 and 20 min. The dye concentrations in the various samples were read against an arbitrary standard containing 4 mg of dyestuff/100 ml. For each test, the 2-min. sample was taken as giving 100 per cent retention and the values for the other samples converted to this scale. The results given in Table I have been expressed as percentage excretion of dye. The above is a modification of the procedure quoted by Lichtman[5]. It was found that the dose rate had little or no effect on the rate of removal of the dye in the normal animals, as is shown by a comparison of the results for normal Southdowns (dose 5 mg/kg) and normal Romneys (dose 2 mg/kg) given in Table I. However, the 5 mg/kg dose resulted in a better definition of the excretion from the normal animal and was chosen for comparing the normal and photosensitive Southdowns.

Serum Alkaline Phosphatase Level

Serum alkaline phosphatase was determined, according to the method of King and Armstrong[6], using a substrate of disodium phenyl-phosphate in a veronal buffer at pH 9.0 and an incubation time of 30 min. at 37.5°C.

Prothrombin Clotting Time

Prothrombin levels are related to the clotting time, which was determined by the method of Quick, Stanley-Brown and Bancroft[7].

Hippuric Acid Synthesis

This test is designed to measure the ability of the liver to conjugate benzoic acid with endogenous glycine to form hippuric acid which is excreted in the urine. The intravenous method of Quick[8] was used. The test animal was catheterized just prior to the intravenous injection of 1.77 g of sodium benzoate dissolved in 20 ml of distilled water. In order to ensure an adequate volume of urine for analysis, it was found necessary to give the animals 1.5 l of water orally at the time of injection. (This also allowed a determination of the water tolerance of these sheep). One hour after injection the sheep was again catheterized and the amount of hippuric acid present in the one hour urine sample determined. Since the sheep normally excretes small amounts of hippuric acid of the order of 0.1 g/100 ml of urine, the pre-injection urine sample was also analysed and allowance made for this in reporting the recovered hippuric acid.

Discussion

The most striking feature of congenital Southdown photosensitivity is the presence of a liver dysfunction with no apparent gross structural abnormality. The liver function tests used in this investigation have been found by other workers to vary greatly in their response after different kinds of liver damage. In the course of the present work an endeavour was made to cover a wide range of functions in order to locate any further deficiencies in the working of the liver of the photosensitive Southdown.

TABLE I. DYE EXCRETION TESTS

(FIGURES GIVEN ARE THE PERCENTAGE EXCRETION OF DYE RELATIVE TO THE 2-MIN. SAMPLE IN WHICH EXCRETION IS TAKEN AS 0% (100% RETENTION)

Sheep*	Body Weight (kg)	SERIAL ROSE BENGAL TEST						SERIAL BROMSULPHTHALEIN TEST						
		Dose	Percentage excretion of dye at stated time (min.) after injection of dose					Dose	Percentage excretion of dye at stated time (min.) after injection of dose					
		ml. of 1% Soln.	2	5	8	12	20	mg/kg	2	4	7	10	15	20
Normal Southdown Ewes														
A 12	65.5	10	0	28.5	54	72	88	5	0	57	68	87	95	100
B 25	43.5	10	0	36	60	79	88	5	0	56	86	93	95	100
17	41.5	10	0	42	66	84	92	5	0	37	81	94	97	100
Photosensitive Southdown Ewes														
S 46	45	10	0	7.5	9	11	16.5	5	0	10	10	20	20	25
S 79	43.5	10	0	11	11	24	24	5	0	0	0	8	17	17
D 485	13	5	0	11	14	17	20	5	0	25	37	37	37	50
Normal Romney Ewes														
104	29	-						2	0	55	82	91	100	-
108	16.5	-						2	0	50	80	90	100	-
117	38	10	0	35.5	54	70	83.5	2	0	42	70	83	92	100
127	31	10	0	40	68	78	93	2	0	54	91	96	100	-
129	33	10	0	39	58	77	87.5	2	0	55	77	95	98	100
179	29	10	0	38	59	75	87	2	0	62	82	100	-	-

* All ewes were 2 years old except D 485 and 108 which were both 1 year old

The dye-excretion tests may be considered together, in view of the similar results obtained with rose bengal and bromsulphthalein (Table 1). The liver of the photosensitive Southdown is unable to remove efficiently either rose bengal or bromsulphthalein from the circulation. Normal Romneys gave excretion curves almost identical

with those of normal Southdowns for both dyes. In view of their similarity in chemical structure, this is not unexpected. Both compounds are phthaleins, but the bromsulphthalein at the pH of blood would be present as the lactone. It is difficult to get an exact comparison of the relative rates of elimination of the two dyes, but bromsulphthalein appears to be removed more rapidly than rose bengal. Rose bengal at a dose rate of 2.3 mg/kg in the normal animal, was still present to the extent of 8-12 per cent of the 2-min. post-injection level, after 20 min. Bromsulphthalein at dose rates of 2 and 5 mg/kg bodyweight was present to the extent of 5-8 per cent after 15 min., while in all normal animals the blood was completely cleared of dye at 20 min. The results of the serial tests on the photosensitive animals appears to indicate that the two-minute sample may be too soon after the injection for thorough mixing of the dye in the bloodstream. The comparatively large fall in concentration between the 2- and 5-min. bleedings is not necessarily due to removal of rose bengal by the liver but more likely, represents continuing dilution with the interstitial tissue fluids. Once uniform mixing is attained, the fall-off in dye concentration due to the inefficiency of the liver mechanism is much slower. Bromsulphthalein appears to be removed from the blood about four times, and rose bengal about ten times, as rapidly in the normal as in the photosensitive Southdown.

This inability to handle phthalein pigments introduced into the circulation may be correlated with Clare's findings[3] of the abnormal presence of porphyrin pigments in the blood and urine of photosensitive Southdowns, and the slight disturbance in bilirubin excretion as evidenced by the positive Van den Bergh reactions.

TABLE II. RESULTS OF LIVER FUNCTION TESTS

Sheep	Plasma Bilirubin (mg/100 ml)	Prothrombin Clotting Time (sec.)	Serum Alkaline Phosphatase (units/100 ml)	Hippuric Acid Excretion after Intravenous Sodium Benzoate		
				Volume of water excreted in 1 hr after 1.5 l given orally (ml)	Hippuric Acid excreted in 1 hr (g)	Calculated yield for complete recovery of dose (g)
Normal Southdowns:						
A 12	--ve	31.0	5.5	290	1.5	2.2
B 26	--ve	32.0	5.3	520	2.1	2.2
17	--ve	35.5	3.8	570	2.1	2.2
Photosensitive Southdowns:						
S 46	0.1	34.5	3.3	98	1.3	2.2
S 79	0.2	36.0	6.3	92	1.5	2.2
D 485	0.1	31.0	2.9	96	0.7	1.1
Normal Romneys:						
108	. .	Mean of 18 determinations 31.7 Range 24-37	Mean of 18 determinations 8.0 Range 3.4-12.1	. .	0.8	1.1
179	. .			. .	1.3	2.2
104	. .			. .	1.6	2.2
117	. .			. .	1.6	2.2
127	. .			. .	1.6	2.2
127	. .			. .	2.2	4.4

An increase of serum alkaline phosphatase is known to occur in obstructive liver disease, which upsets the biliary excretion of the enzyme, while extensive parenchymal damage may interfere with enzyme synthesis and result in a fall in phosphatase level. The results obtained show no appreciable difference between the levels for photo-

sensitive and normal Southdowns, although the values tend to lie toward the lower end of the range found for normal Romneys. (Table II). Allcroft and Folley[9], however, found a very wide variation in serum alkaline phosphatase in apparently normal ewes, so that any fluctuations in the observations above are negligible as far as indicating a liver dysfunction.

The results for the prothrombin clotting times given in Table II show no difference between normal and photosensitive Southdowns, and both these are within the range found for normal Romneys. This would indicate a normal prothrombin level in all the tested sheep which implies adequate absorption of the fat soluble vitamin K and the ability of the photosensitive animal to synthesize normal amounts of prothrombin in the liver.

The hippuric acid excretion test is known to measure the ability of the liver to mobilize and conjugate glycine to form hippuric acid (benzoylglycine) which is excreted in the urine. It has been found by others to be less sensitive than the dye tests and to require normal functioning of the kidney. Moreover, variations in body weight can influence the amount of hippuric acid in the urine. To our knowledge there has been no direct evidence for the synthesis of hippuric acid in the sheep liver. However, the fact that in normal sheep a moderate dose of benzoic acid can be almost completely recovered in the urine as hippuric acid, appears to support the view that the liver is the main synthetic organ. The metabolism of benzoic acid in the dog involves benzoylglucuronic acid as the major excretory product, with some conversion of this to hippuric acid in the kidney.

Working with normal human patients, Quick, Otterstein and Weltchek[10] found that from 1.0 to 1.4 g of hippuric acid was excreted in 1 hour after a dose of sodium benzoate capable to yielding 2.2 g of hippuric acid.

They also showed that hippuric acid synthesis was increased if exogenous glycine was supplied, and interpreted this as an indication that the amount of hippuric acid excreted is a measure of the capacity of the animal for glycine synthesis. It was later found by Quick[8] that the amount of glycine the liver can synthesize and conjugate is limited. Provided that the amount of benzoate dosed is in excess of the ability of the liver to conjugate it all within the given time, the hippuric acid excretion measures the maximal functional capacity of the liver.

The amount of hippuric acid recovered from the Romney sheep after a double dose of benzoic acid, did not exceed 2.2 g—almost the same amount recovered from normal animals given the usual dose. This figure therefore represents the maximal hourly excretion of hippuric acid obtainable from apparently normal sheep. Values obtained for the hourly excretion of hippuric acid from normal sheep ranged from 1.3 to 2.1 g, while those from the photosensitive Southdowns lay nearer the lower limit of the normal range, 1.3 to 1.8 g of hippuric acid, after a dose of 1.8 g of sodium benzoate, which for complete conversion and recovery would yield 2.2 g of hippuric acid. (Table II).

The figures for the volume of urine excreted in 1 hour after the oral administration of 1.5 l of water to the normal and photosensitive Southdowns illustrate the marked delay in the excretion of water by the photosensitive animals. This water tolerance test was incidental to the hippuric acid synthesis and the observed effect could account for the relative inefficiency of the recovery of hippuric acid from the photosensitive sheep.

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VALUE OF THE AWN IN ESTABLISHING SEED OF *Danthonia penicillata* (Labill.) Palisot

By Margaret Simpson, Botany Division, Department of Scientific and Industrial Research, Wellington

(Received for publication, 15 August 1952)

Introduction

After recent broadcast sowings of deawned *Danthonia* seed, experiments were carried out at the Botany Division, Department of Scientific and Industrial Research, Wellington, to determine whether the de-awning process could have a detrimental effect on germination or subsequent establishment.

The "seed" of *Danthonia penicillata* is very light and hairy, and until recently efforts to sow it mechanically have failed. A machine has now been used to remove the hairs and awns from the seed. The advantages claimed for this processing are that it facilitates mechanical feed of seed through hoppers for broadcast sowing from the air and, by reducing the bulk of a given weight of seed, induces a more even spread on the soil surface. It is reported (The Press, Christchurch, 20 October, 1951) that by such cleaning the bushel weight is increased by about four times. This obviously eases transport and increases the seed content of the pay load of both ground transport and aircraft. In the de-awning process hairs are all taken off and awns are cut back to a length of 2 mm or less, all the flexuose tip and part at least of the spirally twisted basal portion being removed.

A sample of de-awned seeds from an aerial sowing trial at Craigieburn, Canterbury, in October, 1951, was used in the experiments. Normal awned seeds of *D. penicillata* grown at the Botany Division nursery at Waiwhetu were used as the control.

Description of Seed

The lemmas are deeply two-lobed at the tip, the lobes being produced into fine points as long as, or longer than, the lemma. A central awn from between the lobes is exserted beyond the floret (Fig. 1). This awn is 8-12 mm long and is flattened and spirally twisted at the base with, usually, three spiral twists. There is a tuft of silky hairs at the base of the pedicel, a tuft on the margin on each side above the pedicel, sometimes connected by hairs on the back, and another marginal tuft on each side opposite to the base of the lobes.

Experimental Procedure

Laboratory Experiments

Petri dish experiments were set up to observe how much movement is caused by alternate straightening and twisting of the awn and to consider the effect that this would have in working seed into the ground.

I. Ten awned and ten de-awned seeds were set at marked intervals on lines on dry filter paper. The paper was then alternately damped and allowed to dry over a period of ten days, and photographs were taken at intervals. Each seed was set with the lemma uppermost and the experiment conducted in a closed room so that there was no draught to cause movement. The dish was kept on a perfectly level surface. The sequence of movements in five adjacent seeds of each kind is illustrated in Fig. 2.

B A

FIG. 1.—A.: Floret of *Danthonia* showing position of awn, hairs and lemma lobes.

FIG. 1.—B: Lower portion of awn with spiral twists.

(*Photographs by A. L. Tilbury*)

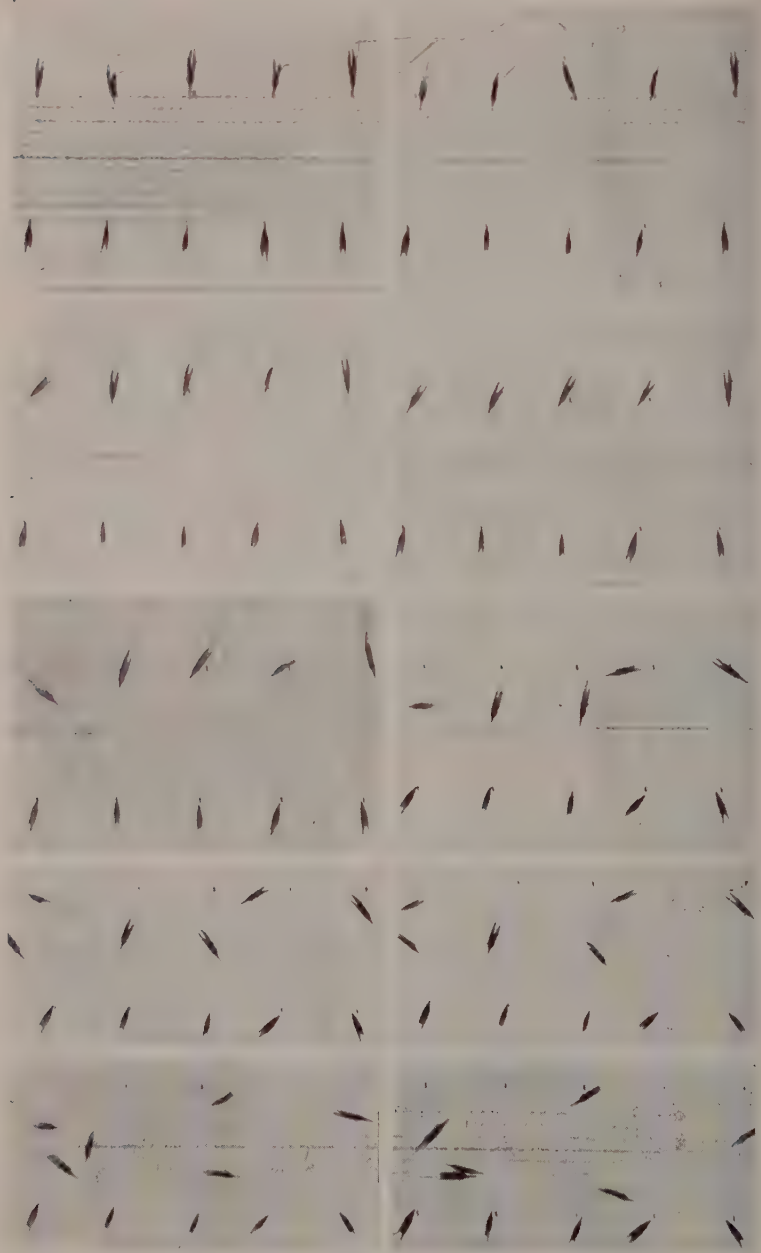


FIG. 2.—"Seeds" of *Danthonia penicillata* showing successive changes in position due to alternate wetting and drying over ten days. Row of seeds originally placed above the ruled lines complete those below deawned. (*Photographs by E. J. Thornley*)

* The blocks in Fig. 2 are designated A to J reading from left to right from the top.

Fig. 2A 19.11.51 10.15 a.m.
Experiment set up on dry filter paper.

Fig. 2B — 11.15 a.m.
Paper was damped and this shows the situation at the end of an hour. The awns have unscrewed, causing each awned seed to turn either on to one side or completely over. Only one de-awned seed has moved and that very slightly.

Fig. 2C — 3.45 p.m.
The paper has begun to dry out and the awns to screw up again.

Fig. 2D 20.11.51 8.45 a.m.
Paper has completely dried out and more movement has been effected by the retwisting of the awn.

Fig. 2E 21.11.51 2.15 p.m.
Paper was redamped as it dried out. This shows further movement 30 hours later.

Fig. 2F 22.11.51 9.00 a.m.

Fig. 2G 23.11.51 9.00 a.m.

Fig. 2H 24.11.51 9.00 a.m.

Fig. 2I 25.11.51 9.00 a.m.

Fig. 2J 26.11.51 9.00 a.m.

In the de-awned seeds the lack of any movement in the direction of the pedicel is very marked and little lateral movement can be detected, the majority having failed to make even half a turn. (In the last four photographs an additional awned seed has moved into the field from the left.)

II. Another experiment was set up exactly the same as the one photographed. Paper was damped at 9.00 a.m. each morning and allowed to dry out during the day. The position of each seed was marked and the change in position recorded daily.

By 1.30 p.m. on the first day five of the awned seeds had made half a turn so that the lemma was facing down, and one had completed a full turn. Besides moving on the long axis, each seed had moved in the direction of the pedicel from 1 to 3 mm with an average of 2 mm. During the following five days the awned seeds continued to move in this same direction about 2 to 5 mm each day and some moved from 1 to 5 mm in a direction at right angles to the long axis of the seed. Nine of the ten seeds turned at least once in this time. In comparison, the de-awned seeds exhibited little movement; only the five with the longest awn remnants moved at all, and the greatest distance covered was barely 2 mm.

Vertical movement, which is not well recorded by either photographs or measurements, was also considerable and important. When the tip of the awn comes in contact with the surface, the action of unscrewing causes the seed to arch so that the lower part of the lemma is inclined to the soil surface at a sharp angle, the pedicel end then being in a position to be forced into any depression

or crevice. With an irregular soil surface this assists the burying of the seed. The upwardly directed hairs then prevent the withdrawal of the seed and subsequent windings and unwindings of the awn cause only further burial.

Outdoor Trial

Two flats were sown outdoors, one with awned and the other with de-awned seeds. The soil was left in a fairly rough condition and the seed scattered over the surface to simulate so far as possible conditions which would prevail after an aerial sowing. A wire netting frame was used to protect seed from birds. Heavy rain fell after the sowing. After three days the awned seed had uniformly inclined itself at an angle to the soil surface and many of these seeds were already pointing into crevices in the soil, whilst the de-awned remained horizontal.

Germination took place after a fortnight, the awned seeds being the more successful. Six months later seedlings from the awned seeds numbered twelve times as many as those from the de-awned.

Conclusion

That the presence of bent or twisted awns and the position occupied by the hairs all assist in burying grass "seed" in the soil has of course long been recognised. The action of these structures in *D. penicillata* has been illustrated in these experiments and the inertia of the de-awned seed compared with th movement of the awned is very striking.

Thus, even if the de-awning process causes no injury to the seed embryo (although injury is a possibility and might account for part of the low germination), the lack of appendages is a distinct disadvantage in establishment, and de-awning of *Danthonia* seed intended for use in broadcast sowing on uncultivated soil surfaces is certainly a procedure which reduces the chances of success.

COMPARISON OF GROWTH OF TOMATO PLANTS IN IMPERVIOUS* PLASTIC POTS AND POROUS CLAY POTS

By J. A. Hunter, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 15 September 1952)

Summary

Comparison of the growth of tomato plants in impervious plastic pots and porous clay pots showed that plants in plastic pots required less water, produced a greater fresh weight of stem and leaves, and developed a more even distribution of roots through the soil mass.

Plastic pots were suitable for growing other plants. They remained free from moss and algal growth, and were easier to clean than the porous clay pots.

Introduction

In the past, flower pots made from many different materials have been compared experimentally with the conventional clay pot which still holds world-wide popularity in practical horticulture.

The assertion that plants make better growth in porous containers has been disputed in horticultural circles for many years. Loudon (1871) and Nicholson (1887) discussed the question and although admitting that some successful cultivators use impervious receptacles, they favoured porous pots. The latter author notes, however, that plants growing in glazed pots required less water.

McHargue (1923) showed that non-porous containers were essential for accurate work when conducting trace-element experiments. From experience and the literature examined, there seems little doubt that many plants will grow satisfactorily in impervious pots.

Preliminary trials were carried out in New Zealand during 1950 to compare the growth of tomato and cauliflower plants in ordinary clay pots and in plastic pots injection moulded from polystyrene. Differences in root distribution and water requirements, demonstrated in these trials, were of sufficient interest to warrant a fuller investigation, particulars of which are given here.

Materials and Method

Three hundred and ten plastic pots and the same number of porous clay pots were used in the experiment. The plastic pots were of two different colours: canary yellow and pale green, and had a soil capacity of 285 g. The difference in colour influenced the amount of transmitted light, but the possible effect of this on root growth was not investigated. Four-inch clay pots were cut to the same soil capacity and were thoroughly soaked in water and allowed to drain before use.

The potting soil and complete fertiliser were the standard ones used by this Division (Hunter, 1952).

*Plastic pots which are claimed to be porous are now being advertised in England. None of these were available in New Zealand when this experiment was carried out.

Single seedlings of Potentate tomato, sown on 4 December, 1950, were planted into each pot on 19 December. All pots then received 100 ml of water which moistened the soil mass without any appreciable loss by drainage, a state which was maintained as far as possible throughout the experiment whenever water was applied. The condition of plant and soil was used to indicate the need and frequency of watering. When it was observed that a number of clay pots required water, all clay pots received an equal volume; a similar procedure was followed with plastic pots. A record was kept of the date and amount of water supplied.

The pots were placed in galvanized trays on benches on both sides of a gabled greenhouse. Equal numbers of clay and plastic pots stood in alternate rows in each tray.

On 16 January every alternate plant in each tray was harvested, leaving 310 pots in the experiment. On 24 January a further 186 were harvested; the final harvest of 124 plants was carried out on 13 February. On each occasion, plants were turned out of the pots, soil removed from the roots by running water, surplus moisture displaced centrifugally and the whole plant weighed individually. At the second harvest a random selection of 25 plants from clay pots and 25 from plastic pots were cut at soil level mark after weighing, and the weight of roots recorded separately. At the final harvest all plants were thus treated.

RESULTS AND DISCUSSION

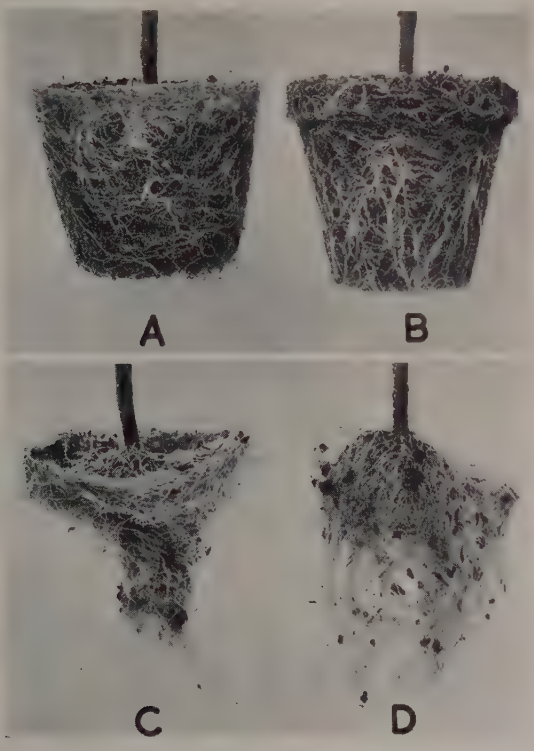
The growth made by the plants in the different containers is recorded in Table I.

TABLE 1. FRESH WEIGHT OF TOMATO PLANTS GROWN IN PLASTIC AND IMPERVIOUS CLAY POTS
Plants potted on 19/12/50 from seed sown 4/12/50

Date of Harvest	Average fresh wt. of plants		Difference for Significance	Aver. shoot wt.		Difference for Significance	Aver. root wt.		Difference for Significance
	Plastic (g)	Clay (g)		Plastic (g)	Clay (g)		Plastic (g)	Clay (g)	
16/1/51	22.9	16.4	1% : 0.9 5% : 0.7						
24/1/51	39.8	34.6	1% : 1.7 5% : 1.3	33.0	27.3	1% : 2.8 5% : 2.1	8.0	7.5	1% : 1.3 5% : 0.9
13/2/51	67.8	64.8	1% : 3.5 5% : 2.7	49.9	47.8	1% : 2.9 5% : 2.2	17.9	16.9	1% : 2.1 5% : 1.6

Table I shows that the weight of stem and leaves of plants from plastic pots exceeded that of plants grown in the porous clay pots. The greatest difference was shown during the first seven weeks of the experiment, but was still significant at the 5 per cent level when the final records were taken. This increase is not the result of a more extensive root system since there was no significant difference in the weight of roots from plants grown in the two types of pot. The average top/root ratio of the plants for the first 36 days of the experiment was 4.12 from plastic and 3.64 from clay. This is close to the figures obtained by Jones and Hoskings (1935) who found that dry-weight top/root ratios for tomatoes grown in glass tumblers was 4.02 while for those grown in porous clay pots it was 3.31.

Fewer roots were visible on the outside of the balls of soil from plastic pots than from clay (Fig. 1 A and B). This had also been noted in the preliminary trial.



The roots of plants in plastic pots were more evenly distributed throughout the soil than those in clay pots in which they tended to be massed at the periphery (Fig. 1 C and D). Matting of roots near the wall is a well-known characteristic of plants grown in porous clay pots. Williamson (1820) discussed the matter and suggested a cultural remedy. Better aeration at the pot margin and the natural tendency for roots to forage outwards from their point of origin have been put forward as the explanation of this phenomenon. Jones and Hoskins (*loc. cit.*) showed that the distribution of roots in plant containers is associated with the distribution of nutrients. They demonstrated that there existed in the soil of porous clay pots an unequal dispersion of soluble soil constituents. Evaporation from the walls is responsible for the lateral movement of moisture and soluble nutrients towards the pot-wall where nutrients are deposited, resulting in a massing of roots in that vicinity.

With the plastic pots there would be little evaporation from the walls with a corresponding absence of lateral movement of water and solutes. This is the probable explanation for the more even dispersion of roots of the plants growing in these containers.

More water was applied to the clay than to the plastic pots. For the first 28 days the plastic pots received only 50 per cent of that supplied to the clay, this figure agreeing with the work of Jones and Hoskings (*loc. cit.*). Towards the end of the experiment differences were less.

Thus, by the 36th day plastic pots had received 61 per cent of the water supplied to clay pots, while by the 56th day the figure for plastic pots was 65 per cent.

The experiment has shown that tomato plants will grow satisfactorily in these plastic pots, and since its conclusion, the pots have been successfully used for other plants. It was observed that no growth of moss or algae appeared on the pot-wall even when used under conditions very favourable to their establishment. These pots are also more easily and quickly cleaned than ordinary clay pots.

The plastic pots used were the only size available in New Zealand at the time of the experiment. They have too small a soil capacity to sustain a quick growing plant for very long, but can, however, be used for growing dwarf succulents, the propagation of some orchids, and possibly any slow growing plant, provided overwatering is avoided.

Acknowledgment

The assistance of H. R. Thompson of the Applied Mathematics Laboratory in doing the statistical analyses is acknowledged.

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NOTE ON THE BREEDING SYSTEM OF *Dichelachne crinita* (Linn.f.) Hook.f. IN NEW ZEALAND

By H. E. Connor and Audrey B. Cook, Botany Division, Department of Scientific and Industrial Research, Wellington.

(Received for publication, 28 May 1952)

Summary

The breeding system of *Dichelachne crinita*, a common perennial grass, has been investigated by comparing the height and vigour of inbred and open-pollinated progenies.

As inbreeding for two generations has not produced any inbreeding depression or deleterious effects in the progeny it is concluded that the species is facultatively self-fertilizing.

Introduction

The small genus *Dichelachne* was erected by Endlicher (1833). *Anthoxanthum crinitum* Linn.f. was transferred to *Dichelachne* by Hooker (1855). Zotov (1943) refers *Dichelachne crinita* (Linn.f.) Hook.f. to *Deyeuxia crinita* (Linn.f.) Zotov, but proffers no reasons for this transfer.

Dichelachne crinita, plume grass, is a perennial, common throughout New Zealand, Australia and Tasmania; it is fully described in Cheeseman (1925).

An attempt to define the breeding system of *D. crinita* is reported in this paper. It has been shown that perennial grasses are, for the greater part, self-sterile (Beddows, 1931; Nilsson, 1934).

In this paper self-fertilization means fertilization of an ovule by pollen of the same plant either from the same flower or from a different flower (autogamy), and cross-fertilization means fertilization of an ovule by pollen from another plant of the same species (allogamy). The term open-pollination is contrasted with self-pollination, to describe plants which are not forced to self-pollinate. Plants which are designated "open-pollinated" may be either cross- or self-fertilized in nature.

Material and Methods

Plants of *D. crinita* were raised from seed of a plant collected from Roxburgh, Central Otago, and growing at the Botany Division Experimental Area, Waiwhetu, Hutt Valley. Several panicles of one plant were selfed in the 1949/50 season. There was no special selection of the plant chosen for selfing. Immature panicles were covered by a 22 in. x 4 in. transparent plastic bag, of the material used for sausage casings, held open by a wire spiral. The top of the bag was folded over and secured to prevent entry of any pollen. The bottom was plugged with cotton wool and firmly tied. The selfing bag was fixed to a supporting stake.

Progeny resulting from seed of this self-fertilization was designated "I_1" i.e. first inbred generation, and open-pollinated plants were given the notation "—9". I_1 progeny and open-pollinated plants were raised in flats. Fifty plants of each generation were planted in a bordered randomised block 10 plants × 10 plants.

In the 1951/1952 season, an I_1 plant was selfed to obtain seed for an I_2 generation. There was no special selection of the plant chosen for treatment. I_2 and open-pollinated progenies were raised as in the previous season.

A further randomised block of 50 plants of each of the I_2 and open-pollinated generations was laid down.

At maturity, the maximum height in centimetres of each plant was measured and a count made of the number of culms produced by each plant. Both these measurements are easily-determined quantitative characters.

As the florets of this species are approximately 5 mm. long, no attempt was made at emasculation.

Results

Results are given in the following table:

Table I. Height of D. Crinita Plants and Number of Culms

Generation	No. of Plants	Mean Height (cm)	Standard Deviation	Mean No. Culms	Standard Deviation
1950/51 Season					
—9	43	80.1 ± 1.0	6.4	23.7 ± 1.8	11.6
I_1	43	79.4 ± 0.9	5.8	27.2 ± 1.9	12.2
1951/52 Season					
—9	50	89.2 ± 1.1	8.0	23.4 ± 1.5	10.7
I_1	50	90.1 ± 1.0	7.4	23.2 ± 1.6	11.3

In neither season were there any visible differences in vigour or any statistically significant differences in height or number of culms between the generations tested.

Discussion

Inbreeding depression is one of the results to be expected when inbreeding is practised on normally cross-fertilizing species. This depression is usually visible in some quantitative character expressed by the plant. No inbreeding depression was visible in inbred progenies of *D. crinita*.

The uniformity of the inbred and open-pollinated progenies indicated that there is no segregation of types; this would be expected in a self-fertilizing species. No chlorophyll deficient plants appeared in the inbred progenies.

As there are known self-fertile forms in species which are normally considered self-sterile (Nilsson, 1934), collections from various localities are now being raised to enable a check on the species as a whole to determine whether this reported instance of self-fertility is common to the species.

If any outcrossing occurs in this facultatively self-fertilizing species, its frequency could be estimated by the use of a marker gene. No suitable gene is yet known in this species. The artificial production of interspecific hybrids would also indicate that outcrossing may occur but this would give only little indication of the frequency of outcrossing within the species, nor would the absence of interspecific hybrids rule out the possibility of such outcrossing.

CONCLUSIONS

D. crinita showed no incompatibility under conditions of self-fertilization. There were no statistically significant differences between plants inbred for one and two generations and those which were continually open-pollinated.

ACKNOWLEDGMENTS

Acknowledgement is made to Mr. G. M. Wright, Applied Mathematics Laboratory, Department of Scientific and Industrial Research, Lincoln, for the statistical analysis of the results, and to Dr. E. J. Godley, Crop Research Division, Department of Scientific and Industrial Research, Lincoln, for his helpful suggestions and criticism.

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SIMPLY CONSTRUCTED GUINEA PIG METABOLISM UNIT, SUITABLE FOR SEMI-QUANTITATIVE DETERMINATIONS OF URINE AND FAECES

By J. V. Evans, Animal Research Station, Department of Agriculture, Wallaceville.

(Received for publication, 27 August 1952)

Introduction

In the course of work on photosensitivity diseases in which the guinea pig was used as an experimental animal, it was necessary to make regular collections of both urine and faeces separately.

Metabolism cages have been described by various authors: Atkin[1], Griffith and Farris[2], Harned *et al*[3] and Kinard *et al*[4], but most of them were designed for use with rats and were not suitable for the present work. A description is given of the design which was eventually found most suitable for use with guinea pigs and which made it possible to handle a number of animals with a minimum of labour.

Design and Construction

Frame: The frame is built up out of $\frac{3}{4}$ in. × $\frac{3}{4}$ in. × $\frac{1}{8}$ in. angle iron and 1 in. × 1 in. × $\frac{3}{16}$ in. T-iron, the basic unit being a 51½ in. × 16½ in. mitred and welded angle-iron oblong frame to which angle-iron cross members are welded on the top side and T-iron cross members on the underside. The angle-iron cross members keep the cages in position and the T-iron cross members suspend the collection cones. This type of construction allows both the cages and the cones to be removed separately without disturbing other members of the unit, thus facilitating handling and cleaning. (See Fig. 1.)

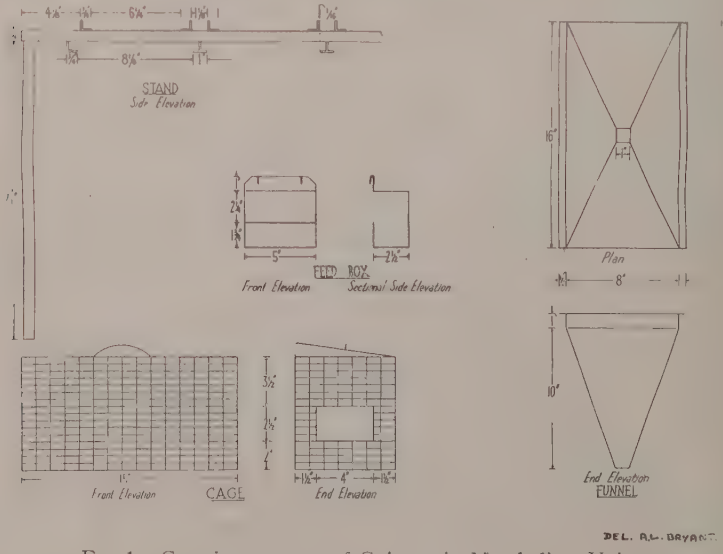

Fig. 1. Constituent parts of Guinea-pig Metabolism Unit.

Cages: The cages are made from 14 s.w.g. galvanised wire with a $1\frac{1}{8}$ in. $\times$ $\frac{3}{8}$ in. mesh, and are 15 in. $\times$ 7 in. $\times$ 4 in. overall. There is a 4 in $\times$ $2\frac{1}{2}$ in. feed port in one end 2 in. from the bottom of the cage. The top of the cage is hinged and forms the door. The cages are designed so that an animal under test can be fed without disturbing the cage. This makes for quicker feeding and less handling.

Feed boxes: These are made from 26 gauge galvanised iron, hold approximately 200 g of dry mash and are attached to the cage over the feed port by two wire clips.

Collection cones: These are constructed from 26 gauge galvanised iron and are designed to slide into the underside of the frame and to centre beneath the floor of the cage above. The inner surfaces of the cones are coated with hard wax.

Separators: Each of these consists of a 60-w electric lamp globe and a 125-ml conical beaker into the neck of which three small projections have been blown. The globes were purchased in an early stage of manufacture and modified by drawing the base to a fine point. The globe rests on the projections in the beaker and a space of approximately $\frac{1}{4}$ in. is then left between the lip of the beaker and the spherical portion of the electric light globe.

OPERATION

Each cage is set up on the frame with a cone suspended beneath it. A separator is placed in a suitable high walled container and set centrally under the 1-in. square hole at the apex of each cone. The bulb of the separator is adjusted so that it is approximately $1\frac{1}{2}$ to 2 in. below the apex of the cone. (Fig. 2.)

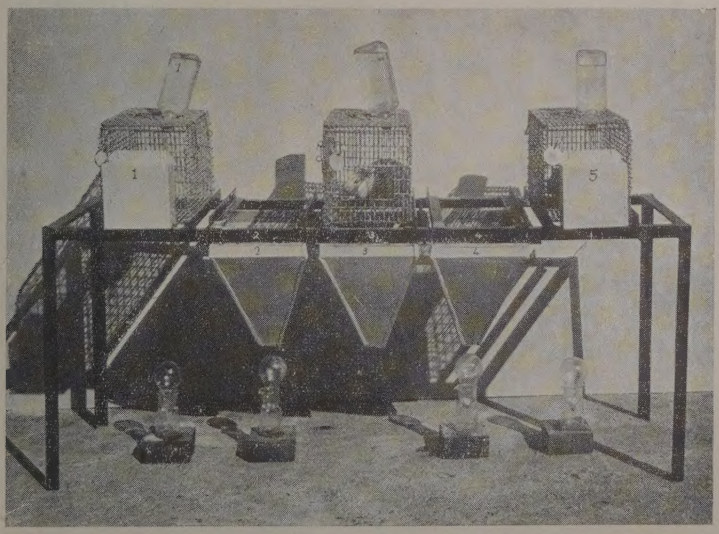

FIG. 2. Guinea-pig Metabolism Unit set up ready for use.

Urine when passed is collected by the cone and drips from the cone on to the bulb, over which it flows to be collected in the 125-ml conical beaker.

Faecal pellets (which in guinea pigs are normally hard) are also collected by the cone and directed on to the bulb, off which they bounce and collect in the high-walled container. The high walls of this container also serve to protect the bulb from draughts, thus reducing the loss of urine by evaporation.

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